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Evaluation of *Juglans regia* L., root for wound healing *via* antioxidant, antimicrobial and anti-inflammatory activity

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The burden of the management of problematic skin wounds characterized by a compromised skin barrier is growing rapidly. There is an urgent requirement for efficient mechanism-based treatments and more efficacious drug delivery systems. The present study was aimed to examine the wound healing potential of *Juglans regia* L. root (JR) in rats by incision and excision wound methods *via* the anti-inflammatory, antioxidant and antimicrobial activities. We have used tensile strength and biochemical parameters for studying the wound healing properties of JR by incision wound methodology. The anti-inflammatory effect was assessed by the measurement of paw edema in carrageenan-induced inflammation in rats. The wound contraction area, antioxidant status, and antimicrobial studies were exhausted excision wound methodology. There was a significant decrease in percent inhibition of paw edema (0.63 ± 0.03 to 0.33 ± 0.02 after 24 h) with an increase in JR concentration. Tensile strength and hydroxyproline level of different concentrations (1, 2.5, 5, and 10% *w/w*) of JR ointment treated groups were found significantly (P <0.001) comparable to the reference group. Moreover, JR showed significant antimicrobial and antioxidant properties, by its ability to increase antioxidant and antimicrobial levels. In conclusion, the overall results obtained in this study clarify that JR inhibits paw edema and accelerates cutaneous wound healing.

Keywords: Cutaneous wound, Paw edema, Tensile strength

Wound healing is a normal biological process that includes inflammation, tissue formation, and remodelling overlapping phases in the human body[1]. It contains soluble mediators, blood cells, parenchymal cells, and extracellular matrix[2]. After the injury, the first stage is the inflammation stage, which causes vasoconstriction that maintains homeostasis and releases inflammation mediators[3]. After that, granulation tissue proliferation formation occurs mainly by fibroblast and the angiogenesis process, which is the characteristic feature of the proliferative phase[4]. The remodelling stage is characterised by reformulations and improvement in tensile strength[5]. Healing is accomplished by the release of eicosanoids, prostaglandins, leukotrienes, and reactive oxygen species (ROS). Among these, ROS plays anessential role in wound healing and serves as primary cellular messengers that constrain several aspects of molecular and cell biology[6]. At the site of injury, ROS is produced in a high amount as a defence mechanism against invading bacteria[7]. At the same time, the process of wound healing may be hindered by the incidence of free radicals, which can damage wound surrounding cells, or by microbial infection[8].

Medicinal plants were revealed to play a vital role in curing skin disorders like cuts and burns. Nevertheless, the selection of plants based on their activity is critical and requires care to determine the value of the plant. Traditional meditative plants are frequently used to get hold of measures beneficial for wound healing purposes comprising an extensive area of various skin-related diseases[9]. Traditionally, *Juglans regia* L. (Family Juglandaceae) has been used for the treatment of cancer, inflammation, diabetes, antiradical, hyperhidrosis, al, and cardiovascular disorders[10–15]. *J. regia* is also a good source of flavonoids, polyphenols, flavonols, carbohydrates, fatty acids, cardiac glycosides, steroids, minerals, tannins, protein, dietary fibre, melatonin, plant sterols, α-tocopherol, folate, tannins, vitamin A and C, and vitamin E family compound[16]. Several studies have reported the antimicrobial activity of phenolic extracts[15-16], making them as best substitute for antibiotics and food preservatives. *J. regia* is a natural product of high economic interest to the food industry and is primarily consumed as royal food worldwide and valued for its nutritional, health, and sensory attributes[16].

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There is widespread interest in using natural antimicrobial compounds due to the growing resistance to antibiotics. Although all parts of this medicinally important plant have been studied for a wide range of pharmacological properties, no study regarding the wound healing property of this part of the plant has been reported yet, as per our best knowledge. Therefore, the objectives of this study were thus to evaluate the antioxidant, anti-inflammatory and antimicrobial potential of ethanol extract of roots of *Juglans regia* L. to find out their wound healing potential using an *in vivo* model.

Materials and Methods

Animals

Wistar albino rats (*Rattus norvegicus*), weighing 160 to 220 g were procured from Tangdu Hospital, Fourth Military Medical University, Shaanxi Province, China. The procedures were reviewed and approved by the Institutional Animal Ethics Committee (Reg. No. 201906-03).

Chemicals and reagents

All the chemicals used during the experiment *viz*., sodium tartrate, copper sulfate, sodium carbonate, hydrochloric acid, chloroform, petroleum ether, ethyl acetate, glacial acetic acid, and perchloric acid were of analytical grade (Sigma Chemical Co., Ltd., USA.). Superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), Folin-Ciocalteu reagent, Ehrlich reagent, and 5,50- dithiobis-(2-nitrobenzoic acid) (DTNB), were purchased from Sigma Chemical Co., Ltd., USA. chloramine-T, Hydroxyproline, carrageenan, and trichloracetic acid (TCA) were purchased from Ranbaxy Pvt., Ltd.

Plant material

The healthy plant root of *Juglans regia* L. (perennial plant)was collected from theQingshui, Mentougou District, Beijing, China during May-June-2018 (Latitude=, Longitude=). The plant materials were dried in the shade, powdered moderate, and pass through a sieve.

Extraction and fractionation

The plant material was shade dried for 3-4 days, ground using an electric blender and the powdered material was then extracted with methanol at 1:3 volumes (1: content and 3: solvent). The supernatant was filtered using Whatman filter paper (Grade:1), and the solution was vacuum evaporated in a rotary evaporator (Heidolph, Germany). The crude extracts of roots of *Juglans regia* L. (JR) were stored at 4°C in the refrigerator until further assay.

Experimental protocol and preparation of test sample

The extract was developed in the ointment by fusion technique, using simple ointment base BP. The different groups of animals were treated by applying extract ointment and iodine ointment. Six animals were kept in each group for studying different parameters. The Group I was referred to as a control group, which received only a simple ointment base. The Group II, III, IV, and V received JR faction ointment with 1%, 2.5%, 5%, and 10% *w/w*, respectively. Group VI denoted as a reference group that received 10% *w/w* iodine ointment. The healing property was assessed in terms of physical and biochemical parameters.

Wound healing activity

Incision wound creation

A 2 cm long incision was made in all the animals through their skin at the dorsal portion. The animals were anaesthetized before wound creation was performed, and no antimicrobials were used throughout the experiment. Both wound edges were tightened for the nice closure of the wound, and once sewing, the injury was left undressed. All extract and reference drug ointment were applied daily up to 12 days; once damages were recovered, the sutures were removed on the 12^{th} day, and with the help of tensiometer, the tensile strength of cured wound skin was measured.

Excision wound creation

An excision wound was inflicted with the aid of reducing away a 300 mm^2 full thickness of skin from a derived space and left undressed to the open environment. After wound formation, wound contraction was measured as percent contraction in every two days. The injuries were left undressed to the free atmosphere and determined daily. The treatments were applied locally second every day, beginning from the wound induction until the complete restoration to cowl all wounds. Small skin samples were amassed on the 20^{th} day, and biochemical estimation and the histopathological study were performed.

Wound breaking strength measurement

Breaking energy is the resistance to breaking below tension that suggests how a great deal the repaired tissue resists breaking below anxiety. It indicated the restored tissue condition. Before checking out, the animals were anaesthetized with an open mask. The sutures had been eliminated from the stitched wounds

of rats after recovery, and tensile energy become measured. The newly repaired tissue, including scar, became used to degree the tensile power by the usage of the tensiometer. The tensile strength increment indicates higher wound healing stimulation using the carried out drug treatments.

Percent wound contraction and epithelialization time

The wound margin was traced at two days intervals after wound creation on transparent graph paper having a millimetre scale that became measured *via* a calliper with an accuracy of 1/20 mm measurements that have been endured up to finish recuperation. The healed area was then calculated by subtracting the initial wound area to the unhealed area after each two days interval. The contraction was represented as percent wound contractions, and epithelialization time was observed after the complete restoration. The rate of healing expressed as percentage contraction:

$$\text{Percent wound contraction} = \frac{\text{Healed area}}{\text{Total area}} \times 100$$

Protein estimation

The protein content material of skin tissues was determined on the post-wounding day 20th. The tissue lysate became dealt with an aggregate of sodium tartrate, copper sulfate, and sodium carbonate. The mixture was left to stand for 10 min and then treated with Folin-Ciocalteu reagent that ended in a bluish shade in 20-30 min. The absorbance was recorded by using a Spectrophotometer at 650 nm.

Collagen content measurement

The hydroxyproline content of wound tissues, which is a basic constituent of collagen, had been analysed on the 20th day. Hot air oven-dried tissues at 60-70°C to constant gain weight were hydrolysed in 6 N HCl at 130°C for 4 h in sealed tubes. The hydrolyzate was then neutralised (pH 7) and subjected to Chloramine-T oxidation for 20 min. However, the reaction was terminated by the addition of 0.4 M perchloric acid, and the colour developed due to the Ehrlich reagent at 60°C was read at 557 nm by using UV Spectrophotometer (Agilent Technologies, USA).

Enzymatic and non-enzymatic antioxidant assay

The skin tissues collected from all treated animals were used for analysing the antioxidant activity. First, these tissues have been homogenised in phosphate buffer (pH, 7.0) and centrifuged under the cold situation. The clear supernatant was then used to study the antioxidants level. Based on the inhibition of epinephrine auto oxidation by the enzyme, the SOD level was recorded. CAT was estimated following the breakdown of hydrogen peroxide. The reduced GSH level was determined by instantly precipitate out homogenates mixture with 25% TCA (0.1 mL), and after centrifugation, the precipitate was removed. By the addition of 0.6 mM DTNB (2 mL) and 0.9 mL of 0.2 mM sodium phosphate buffer (pH 8.0) to the supernatant (0.1 mL), free-SH groups were assayed, and the absorbance was recorded at 412 nm in UV spectrophotometer.

Anti-inflammatory activity

Carrageenan-induced rat paw edema model was used for studying the anti-inflammatory activity on albino rats. The prepared fractions were applied by gently rubbing to the plantar surface of the left hind paw and inflammation was induced by subplantar injection of 0.1 mL of 1% carrageenan solution after one hour in normal saline into the treated paw of all rats. The paw volume of each rat was measured by using a plethysmometer in millilitre up to 24 h post-carrageenan injections. The percentage of anti-inflammatory activity was calculated using the following equation:

$$\text{Percentage inhibition} = \frac{(Vc - Vt)}{Vc} / \times 100$$

where, Vt is the paw volume of the test group, and Vc is the paw volume of the control group.

Antimicrobial activity

The antimicrobial activity of JR was evaluated against two Gram-positive bacterial strains *viz*. *Pseudomonas aeruginosa* and *Staphylococcus aureus*, two Gram-negative bacterial strains *viz*. *Escherichia coli* and *Proteus mirabilis*, and against two fungal strains *viz*. *Candida albicans* and *Candida glaborata*. The stock cultures were maintained on Muller–Hinton Agar (for bacteria) and Sabouraud dextrose agar medium (for fungi) at 4°C.

Disc diffusion assay and determination of Minimum Inhibitory Concentration (MIC)

The disc diffusion method was used for studying the antimicrobial activity of JR. The susceptibility tests were performed on Muller–Hinton Agar (bacteria) and Sabouraud Dextrose Agar (fungi). Different concentrations *viz*., 50, 25, and 12.5 mg (diluted with mg/mL 5% dimethyl sulfoxide (DMSO) of all extracts were impregnated on the filter paper discs (6 mm) during the study. Ciprofloxacin (5 mg/disc) and Amphotericin B (20 mg/disc) were used as positive reference standards for bacteria and

fungi to determine the sensitivity of the tested strains. 5% DMSO was used as blind control. Finally, the plates were incubated at 37°C for 24 h (for bacteria), 28°C for 48 h (for fungi), and the inhibition zones were observed including the diameter of the disc (6 mm).

Statistical analysis

Pharmacological data were represented as the mean ± standard error of the mean for six replicates and data were evaluated using the Turkey test. Values of P <0.01 were considered to be statistically significant when analyzed by using ANOVA.

Results

Wound healing activity

Wound breaking strength measurement

The tensile strength shows how resistant the repaired tissue is to break under tension and can indicate the quality of the repaired tissue[17]. The present study shows the tensile strength measurement on day 12 (Table 1). It was found that the tensile strength of the JR treated animals was significantly greater than the control group and comparable to the reference group.

Determination of percent wound contraction

Wound contraction involves the wound being contracted, narrowed or closed. The healing process is regulated by collagen synthesis, deposition, and maturation[20]. The measurement occurs every two days after extracts were applied until the wound was completely healed. On day 6–12 treated wounds with 10% *w/w* of JR showed a significant increase in the percentage of wound contraction compared to the control group (Table 2). A significant difference in the percentage of wound contraction was observed in the group treated with JR from 14 to 20 days compared to the control group and comparable to the reference group.

Protein estimation and Collagen content measurement

The protein content of granulation tissues indicates the level of protein synthesis and cellular proliferation[22]. During the present study, the group of animals treated with 10% *w/w* of JR (81.23 ± 2.01) and a reference ointment (84.16 ± 2.22) found significantly higher protein content than the control groups (35.11 ± 3.04) (Table 3). The high protein content of treated animals suggests that through an unknown mechanism, the JR stimulates cell proliferation. An increase in protein content is caused by an increase in collagen synthesis[23]. In the

Table 1 — Effect of *Juglans regia* L. on tensile strength of incision wound

Experimental Groups	Breaking strength (gm/cm^2)
Control	397.7 ± 7.11*
JR (1% *w/w*)	398.7 ± 6.31
JR (2.5% *w/w*)	578.6 ± 13.32
JR (5% *w/w*)	769.9 ± 9.82
JR (10% *w/w*)	999.8 ± 7.95**
Iodine Ointment	1012.4 ± 14.25

n=6 per group, the value represents mean ± SEM (Standard error of the mean). *P<0.001, **P <0.01, when compared each treated group with the control group.

Table 3 — Effect of *Juglans regia* L. on hydroxyproline and protein content of tissues from incision wound

Experimental Groups	Hydroxyproline content (mg/g tissues)	Protein content (mg/g tissues
Control	29.12 ± 2.97*	35.11 ± 3.04*
JR (1% *w/w*)	40.47 ± 3.13	55.39 ± 4.18
JR (2.5% *w/w*)	45.96 ± 2.69	67.17 ± 3.90
JR (5% *w/w*)	65.99 ± 5.10	73.02 ± 2.46
JR (10% *w/w*)	79.18 ± 3.91**	81.23 ± 2.01**
Iodine Ointment	73.00 ± 3.71	84.16 ± 2.22

n=6 per group, the value represents mean ± SEM (Standard error of the mean). *P< 0.001, **P<0.01, when compared each treated group with the control group.

Table 2 — Effect of *Juglans regia* L. on epithelialization period of excision wound and percentage wound contraction

Experimental Groups	Post wounding days										Epithelialization period
	2	4	6	8	10	12	14	16	18	20	
Control	10.33±1.13	17.85±1.11	27.18±2.33	36.85±1.77	39.21±2.12	47.69±2.95	52.12±3.75	54.93±2.99	59.18±4.10	63.01±2.95	30
JR (1% *w/w*)	11.89±1.21	22.78±1.48	34.90±2.04	43.70±2.90	45.28±1.19	49.14±3.71	54.13±2.81	61.71±3.37	66.99±2.77	70.23±3.02	28
JR (2.5% *w/w*)	13.01±1.44	24.12±1.98	37.67±4.22	45.12±2.11	49.58±2.99	56.67±2.32	59.19±3.03	68.50±4.03	76.55±3.11	80.15±3.27	26
JR (5% *w/w*)	14.99±1.92	28.18±2.19	41.58±4.97	48.11±2.46	59.60±1.95	63.80±3.75	67.89±4.14	77.97±3.31	82.19±2.11	88.88±3.20	23
JR (10% *w/w*)	16.05±3.11	31.72±3.29	44.49±3.79	56.16±5.19	63.12±3.68	70.35±3.76	79.72±3.25	87.81±4.32	95.86±4.78	98.01±2.08*	20
Iodine Ointment	17.33±2.13	32.12±2.39	47.89±5.90	60.18±4.99	69.20±5.01	75.88±3.12	82.42±4.76	90.53±3.17	97.18±4.26	102.11±3.17	20

n = 6 animals per group, the tabular value represents mean ± SEM (Standard error of the mean). *P <0.01 as compared to group control and reference at particular days.

present study, the total protein content of treatment groups has been increased; it implies that the treatment stimulates cell proliferation. Wound strength depends on collagen remodelling and establishing stable intra and inter-molecular cross-connections. Large fibrils and complex fibrous superstructures are made of collagen that is primarily responsible for the tensile strength of the tissue[26]. As a result, hydroxyproline measurement was used as an index of collagen turnover. The content of hydroxyproline was determined in the small tissue specimen collected from each group of animals. The level of hydroxyproline in a group treated with JR 10% *w/w* was found to be significantly increased (Table 4).

The enzymatic and non-enzymatic antioxidant of JR

Cutaneous wounding causes depression in the entire antioxidant status, making it more vulnerable to radical oxygen attack. All of these findings show that antioxidants can play an essential role in wound healing. Our results also show that a significant decrease in antioxidant levels was observed in skin tissues, in the animals treated with JR ointment compared to the reference group. The slightly increased concentration-dependent levels of SOD, CAT, and GSH were found in the JR ointment treatment group. On complete healing, JR (10% *w/w*) level of antioxidants (SOD and GSH) and animals treated with reference ointment were significantly improved. A higher level of SOD and CAT was also shown in the treatment group JR (10% *w/w*). This property may be associated with its composition, which includes the presence of beneficial antioxidants.

Anti-inflammatory activity of JR

In carrageen-induced paw edema in rats, the anti-inflammatory effects have been observed to increase significantly in the JR ointment group (10% *w/w*) and are comparable to the reference group at 4 to 6 h after induction (Table 5), this may be due to its antioxidant activity.

Anti-microbial activity of JR

The antimicrobial potential of JR against two Gram-positive and Gram-negative bacteria and two fungi were evaluated by using the disc diffusion method and determination of minimum inhibitory concentration, and the results are presented in (Table 6). The results revealed that JR showed significant antimicrobial activity against all the bacterial and fungal strains tested. The mean zone of inhibition produced by the extract ranged from 9.3 ± 0.57 to26.2 ± 0.50 mm, and the MIC values were in between 7.81 and 31.25 mg/mL.

Table 4 — Effect of different Concentrations of *Juglans regia* L. on antioxidants level of tissues from excision wound

Experimental Groups	Enzymatic and non-enzymatic antioxidant levels		
	SOD (mg/50 mg tissue)	CAT (mmol/50 mg tissue)	GSH (mmol/50 mg tissue)
Control (Base)	16.19 ± 0.55*	11.18 ± 0.19*	22.96 ± 0.92*
JR (1% *w/w*)	21.67 ± 1.52	17.01 ± 1.05	24.01 ± 1.01
JR (2.5% *w/w*)	24.95 ± 1.99	23.14 ± 0.55	28.19 ± 1.04
JR (5% *w/w*)	29.41 ± 1.98	24.97 ± 0.89	30.33 ± 1.11
JR (10% *w/w*)	31.67 ± 1.50**	27.67 ± 0.81**	32.51 ± 1.71**
iodine Ointment	30.11 ± 1.43	27.13 ± 1.21	35.07 ± 2.94

n=6 Animals per group, the value represents mean ± SEM (Standard error of the mean). *P< 0.01, **P < 0.001, when compared each treated group with the control group in respective parameters. SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione.

Table 5 —Effect of *Juglans regia* L. on carrageenan-induced rat paw edema

Experimental Groups	Carrageenan-induced rat paw edema means ± SEM (percentage inhibition of paw volume)				
	1 h	4 h	6 h	8 h	24 h
Control	0.70 ± 0.02	2.06 ± 0.17*	0.99 ± 0.07	0.74 ± 0.02	0.63 ± 0.03
JR (1% *w/w*)	0.62 ± 0.03	1.91 ± 0.06	0.90 ± 0.03	0.68 ± 0.03	0.55 ± 0.02
JR (2.5% *w/w*)	0. 60± 0.04	1.00 ± 0.03	0.79 ± 0.04	0.63 ± 0.02	0.47 ± 0.01
JR (5% *w/w*)	0.50± 0.05	0.98 ± 0.03	0.61 ± 0.03	0.56 ± 0.03	0.39 ± 0.02
JR (10% *w/w*)	0.42 ± 0.02	0.85 ± 0.03**	0.53 ± 0.01	0.48 ± 0.01	0.33 ± 0.02
Iodine Ointment	0.35 ± 0.01	0.72 ± 0.02	0.47 ± 0.01	0.40 ± 0.02	0.25 ± 0.01

n = 6 animals per group, the tabular value represents mean ± SEM (Standard error of the mean). **P<0.01 as compared to group control and reference at particular days.

Table 6 —Antimicrobial activity of *Juglans regia* L. against bacterial and fungal strains

S. No.	Microorganisms	Mean zone of inhibition[a] (mm)[b]			Ciprofloxacin (5 µg/disc)/ Amphotericin B (20 µg/disc))	MIC (µg/mL)	MBC/ MFC (µg/mL)
		Concentration of the JR (µg/disc)					
		50	25	12.550			
1.	Pseudomonas aeruginosa	15.1 ± 0.28	11.3 ± 0.57	9.3 ± 0.57	13.0 ± 0.5	31.25	62.5
2.	*Staphylococcus aureus*	19.1 ± 0.28	15.0 ± 0.50	13.0 ± 0.50	14.3 ± 0.57	31.25	62.5
3.	*Escherichia coli*	18.0 ± 0.50	15.0 ± 0.50	12.1 ± 0.28	28.1 ± 0.28	31.25	62.5
4.	*Proteus mirabilis*	16.3 ± 0.50	13.6 ± 0.76	10.8 ± 0.76	27.0 ± 0.50	31.25	62.5
5.	*Candida albicans*	26.2 ± 0.50	19.3 ± 0.76	14.5 ± 0.76	26.6 ± 0.76	7.81	15.62
6.	*Candida glaborata*	24.1 ± 0.28	19.0 ± 0.50	13.3 ± 0.57	25.0 ± 0.57	15.62	31.25

[a]Diameter of the zone of inhibition (mm) including the disc diameter of 6 mm
[b]Mean of three assays; ± - Standard deviation

DISCUSSION

Wound healing is a complex process of restoring cellular structures and tissue layers in damaged tissue together to its normal state and commencing in the fibroblastic stage where the area of the wound undergoes shrinkage. Collagen provides tensile strength and elasticity to the healing skin[18]. An increase in the collagen content of the treated injuries corresponds to the significantly increased tensile strength of both treated groups of incision wounds compared to that of the untreated wounds[19]. The tensile strength of an injury can, therefore, be associated with its formation and collagen maturation. Wound contraction involves the wound being contracted, narrowed or closed. The healing process is regulated by collagen synthesis, deposition, and maturation[20]. JR treated group showed a faster contraction of the wound might be due to anti-inflammatory activity that includes, interleukin-8 stimulation, an inflammatory α-chemokine which changes the function and recruitment of different inflammatory cells, fibroblasts, and keratinocytes[21].

From a clinical point of view, collagen deposition in the wound is an essential phase of healing. It was found together to improve healing, facilitate oxygen diffusion, decrease oxygen-free radical overproduction, and increase collagen synthesis[24]. Fragments derived from collagen and collagen regulates various cellular functions such as cell shape and differentiation, migration and integration of several proteins in the extracellular matrix[25]. Wound strength depends on collagen remodelling and establishing stable intra and inter-molecular cross-connections. Large fibrils and complex fibrous superstructures are made of collagen that is primarily responsible for the tensile strength of the tissue[26]. As a result, hydroxyproline measurement was used as an index of collagen turnover. The content of hydroxyproline was determined in the small tissue specimen collected from each group of animals. The level of hydroxyproline in a group treated with JR 10% *w/w* was found to be significantly increased (Table 3). This property may be due to its antioxidant activity and flavonoid composition since flavonoids have been shown to increase collagen synthesis, support collagen cross-link and decrease soluble collagen degradation[27].

Oxidative stress is responsible for the production of reactive oxygen species (ROS), resulting in pathogenesis like diabetes[28] and delayed healing process and cytotoxicity[29]. Neutrophil-derived peroxidases contribute to tissue damage during chronic wounds, along with oxidative stress[30]. Therefore, the primary strategy to heal the chronic wound is to eliminate the ROS that has been produced over. Our results also show that a significant decrease in antioxidant levels was observed in skin tissues, in the animals treated with JR ointment compared to the reference group. These results are comparable with the previous reports of reducing oxidative stress by *Terminalia arjuna* against acetaminophen-induced hepatotoxicity in Wistar albino rats[31] and the results of *Trigonella foenum-graecum* against diabetes induced oxidative DNA damage[32].In three phases wound healingcan be discussed, the aspect of inflammation, proliferation, and maturation or remodelling. Haemostasis and inflammation characterise the inflammatory phase. Epithelialization, angiogenesis, and deposition of collagen follow the proliferative phase[33]. The wound undergoes contraction during the maturation phase, resulting in a smaller amount of visible scar[34]. In carrageen-induced paw edema in rats, the anti-inflammatory effects have been observed to increase significantly in the JR ointment group by 10% *w/w* and are comparable to the reference group at 4 to 6 h after induction.

Antimicrobial agents also provide better and quicker healing by forming a barrier against microbial contamination. The damaged skin remains vulnerable to all kinds of invasive microbial infections that can lead to wound inflammation and fluid exudation, interfering with the process of healing[35]. The results showed that all of the formulations tested had excellent antibacterial activity against Gram-negative and Gram-positive bacteria, the pathogens usually involved in skin infections. It can, therefore, be concluded that by preventing and managing wound infections, the antibacterial activity of the tested extracts could contribute to the creation of an appropriate environment for wound healing. Interestingly, the extracts being investigated showed significant antibacterial activity in comparison with reference drugs known for their actions.

Although the exact mechanisms and modes of action of the JR described in this work have not yet been entirely determined, their wound healing effects could probably be attributed to their bioactive molecules and their associated antimicrobial, antioxidant and anti-inflammatory activities. These properties provide further evidence to support *Juglans regia* L. root's beneficial pharmacological effects in wound healing.

Conclusion

The findings of this study indicated that *Juglans regia* L. root (JR)'s topical application accelerated wound contraction and had beneficial effects on skin wounds in rats that were experimentally induced. The findings also showed significant antioxidant, anti-inflammatory, and antimicrobial properties of the JR. The results supported *Juglans regia* L. leaf's promising candidacy for application as a therapeutic agent for dermal wound healing that appears to be associated with its antioxidant, anti-inflammatory, and antimicrobial properties. Accordingly, additional studies are needed to further investigate the optimal conditions for the production and application of this promising *Juglans regia* L. root.

Conflict of interest

All authors declare no conflict of interest.

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