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WOUND HEALING POTENTIAL OF METHANOLIC EXTRACT OF *Juglans regia*ON ALBINO RATS

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ABSTRACT:

Information from ethnic groups or indigenous traditional medicine hasplayed a vital role in the discovery of novel products from plants as chemotherapeuticagents. Wound healing is a complex and complicated process. It runs through a number of phases, which either run concurrently or are intimately interlinked through some chemical, biochemical and cellular pathways.Juglans regia is a medicinal plant which is widely used for the treatment of many ailments.The array of human health benefits derived from this plant is due to the abundant presence of the different phytochemical compounds present in it. Plant material (leaves) of Juglans regia of family Juglandaceae was collected from Saloora Ganderbal, Kashmir. The preliminary phytochemical screening of the plant material was performed which showed the presence of many phytochemical compounds in it. The extracts were formulated 1% w/v in gum acacia dissolved in normal saline. JR (Juglans regia) 1% w/v was used as dose. A full thickness of the excision wound (400mm^{2}appx.) was created along the markings using toothed forceps, a surgical blade and pointed scissors.It was observed that wound contractingability of animals treated with ointment containing 1% (w/w)methanolic extracts of Juglans regia were found to be significantly higher ($P < 0.001$)on days 8th, 12th and 16th as compared to the control group.

KEY WORDS: Juglans regia, ailments, phytochemical analysis, excision wound, methanolic extracts.

1. INTRODUCTION:

Herbal medicines have been enjoyed revitalization among the clients all over the world. There are hundreds of medicinal plants that have a long history of curative properties against various

diseases and ailments. However, screening of plants for their activity is very crucial and needs imperative attention in order to know the value of the plant.Information from ethnic groups or indigenous traditional medicine hasplayed a vital role in the discovery of novel products from plants as chemotherapeuticagents (Katewa *et al*., 2004).According to WHO (World Health Organization), 70% population of the world depend on Traditional Health Care System (THCS) for curing various diseases (WHO, 2002).

A wound can be defined as a break in the continuity of the soft tissues like skin, mucous membranes, tissue surfaces etc. caused by physical, chemical or biological insult. Wound can also be called as a traumatic lesion.Wound healing is a complex and complicated process. It runs through a number of phases, which either run concurrently or are intimately interlinked through some chemical, biochemical and cellular pathways.The process ofwound healing occurs in four phases: (i) coagulation,which prevents blood loss, (ii) inflammation and debridementof wound, (iii) repair, including cellular proliferation, and (iv) tissue remodeling and collagen deposition (Puratchikody et al., 2006).

Wound healing takes place in several stages like inflammation, proliferation & remodeling. Several studies on plants used in wound healing have been carried out. Srivastava & Durgaprasad (2008) has shown the effect of *Cocus nucifera* on burn wound healing. Gupta et.al. (2006) have reported effect of *Rhodiola imbricata* on dermal wound healing. Nayak et.al. (2007) has reported wound healing activity ethanolic extract of *Morinda citrifolia*. Nayeem et.al. (2009) have reported wound healing activity of alcoholic extract of *Ficus religiosa*.

Juglans regia family*Juglandaceae,*is a large deciduous tree attaining heights of 25–35 m, and a trunk up to 2 m diameter, commonly with a short trunk and broad crown, though taller and narrower in dense forest competition. It is a light-demanding species, requiring full sun to grow well.The leaves are alternately arranged, 25–40 cm long, odd pinnate with 5–9 leaflets.*Juglans regia* leaves have been used mostly in worldwide traditional medicines as antimicrobial, antihelmintic, astringent, keratolytic, antidiarrhoeal, hypoglycaemic, depurative, tonic, carminative, and for the treatment of sinusitis, cold and stomach ache.

2. MATERIALS AND METHODS:

2.1. Plant material:

Fig 1: *Juglans regia*Plant.

Plant material (leaves) of *Juglans regia* of family Juglandaceae was collected from Saloora Ganderbal, Kashmir (2012). The specimen of the plant for the study was identified in the department of taxonomy of Kashmir University and the herbarium was deposited in the same department as a record under the specimen no. 1716 KASH for *Juglans regia*. The plant material was washed thoroughly with water and then air dried in shade at room temperature 25 ± 2°c for more than 15 days. The air dried plant material was grinded to powder about 40 – 60 mesh size.The 50gm of the powdered material was loaded into soxhlet apparatus separately for extraction with the solvent (Methanol). The extract was filtered throughWhatman's filter paper. Then the crude extract was concentrated in the vacuum rotary evaporator. The extract obtained from plants was tested for wound healing in rats.

2.2. Preliminary phytochemical screening:

Preliminary phytochemical investigation of the selected plant materials were done using various phytochemical tests including Dragendroff and Mayer's tests for alkaloids, alkaline reagent test for flavonoids and Kellar-Killiani test, Froth formation test, Salkowski test for cardiac glycosides, glycosides saponins, and steroid-terpenoid, respectively. Alkaloids, flavonoids, saponin glycosides, steroids and terpenoids were found strong positive in *Juglans regia.*

2.3. Preparation of formulation:

The extracts were formulated 1% w/v in gum acacia dissolved in normal saline. JR (*Juglans regia*) 1% w/v was used as dose.

2.4. Animal material -Wistar albino rats:

The study was conducted on male albino rats of Wistar strain (*Rattusnorvegicus*). The rats (120-200gms) were obtained from the animal house of Pest Control and Ayurvedic Drug Research Laboratory, S.S.L. Jain College Vidisha (M.P.). The experimental work was carried out under the supervision of IAEC as per the guidelines of CPCSEA (Reg. no. 804/03/ca/CPCSEA) and maintained under controlled conditions at temperature of 22±2°C, humidity 60±10% and a 12h light/dark cycle. They had free access to standard rodent pellet diet (Golden Feeds Private Ltd., Badodara) and water *ad-libitum.* Animals were periodically examined before and after the experiment. The rats were anaesthetized prior to during infliction of the experimental wounds. The surgical interventions were carried out under sterile conditions using Ketamine anesthesia (80mg/kg body). Animals were closely observed for any infection and if they showed any signs of infection were separated, excluded from the study and replaced.

2.5. Study Design:

For each experimental model the animals were divided into groups of four animals each.

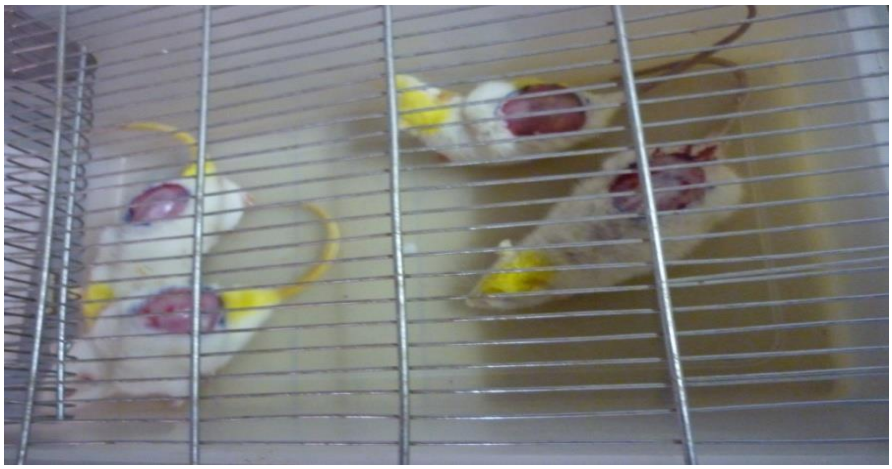

Fig 2: Wistar Albino Rats for study design

Experiment

Group I served as control - received vehicle topically.

Group II served as standard - received betadine 0.2% w/v.

Group III received 1%w/v herbal formulation of *Juglans regia.*

The study was carried out on Ketamine anaesthetized rats in excision wound model.

Excision wound model:

The rats were inflicted with excision wounds as described by Morton and Malone (1972). The dorsal fur of the animal was shaved and the area of wound to be created was outlined on the back of animal with methylene blue. A full thickness of the excision wound (400mm^{2}appx.) was created along the markings using toothed forceps, a surgical blade and pointed scissors. The parameter studied was wound closure, epithelialization time and collagen content. The measurement of the wound area was taken on every fourth day.

Statistical Analysis:

Results are expressed as mean±stdev. The comparisons between experimental groups were made using one way ANOVA fallowed by Bonniferon's test.

Histological Studies:

Thesample tissues formed on excision wounds were excised on 16th dayand washed thoroughly in Bouin's solution. They were fixed in 10% formalin solution for 24hrs.There after the material was washed in tape water and dehydrated in alcohol series, cleared in xylene and transferred to the molten paraffin wax of 60ºC – 62º C in the oven. After 3 changes of half an hour duration the blocks were prepared using metal blocks and paraffin blocks were cut at 5-7µ Thickness by the Rotatory microtome. The stretched slides were kept in slide box for staining in toluidine blue. The slides were examined under high power microscope and the process of re-epithelization was observed.

3. RESULTS:

3.1. Extraction of Plant Material

Extraction and isolation of the plant materials were done using Soxhlation and cold percolation of selected plant material which was used in powdered form of 40-60 mesh size. For the soxhlation the powdered material of the plant material kept in the soxhlet apparatus, then ethanol and distilled water were preferred for extraction. The percentage yield of plant material of *Juglans regia* extract after soxhlation were obtained 8.57 % in ethanol and 10.75% in water.

Similarly, the percentage yield of crude extract of plant material obtained by cold percolation in ethanol and distilled water solvents preferred. The percentage yield of whole plant of *Juglans regia* extract were obtained 5.00 % in ethanol and 9.90 % in water.

Table 1: Percentage yield of crude extract of plant materials using Soxhlet apparatus

S. No.	Name of the Plant	Weight of dry material(gm)	Solvent used	Volume of Solvent	Weight of Plant Extract(gm)	Yield (%)
01	*Juglans regia*	200gm	Ethanol	1000ml	17	8.57
		200gm	Distilled water	1000ml	21.5	10.75

Table 2: Percentage yield of crude extract using Cold Percolation

S.No.	Name of the Plant	Weight of dry material(gm)	Solvent used	Volume of Solvent	Weight of Plant Extract(gm)	Yield (%)
01.	*Juglans regia*	200gm.	Ethanol	1000ml.	10	5.00
		200gm.	Distilled water	1000ml.	19.8	9.90

Fig 3: Extraction of *Juglans regia*leaves

3.2. EXPERIMENTAL BIOASSAY ON WOUND HEALING

The results of wound healing activity by excision wound modelare presented in Table below. The size of wound contraction of each animal in each group has been shown in tables. The values presented in the tables represent wound contraction of each group at 0th, 4th, 8th, 12th and 16th days, for control(simple ointment B.P. treated group.), standard (Betadinetreated group) and the test groups viz. the methanolic extract of *Juglans regia*(1%, w/w). It was observed that wound contractingability of animals treated with ointment containing 1%

(w/w)methanolic extracts of *Juglans regia* werefound to be significantly higher ($P < 0.001$)on days 8th, 12th and 16th as compared to the control group.

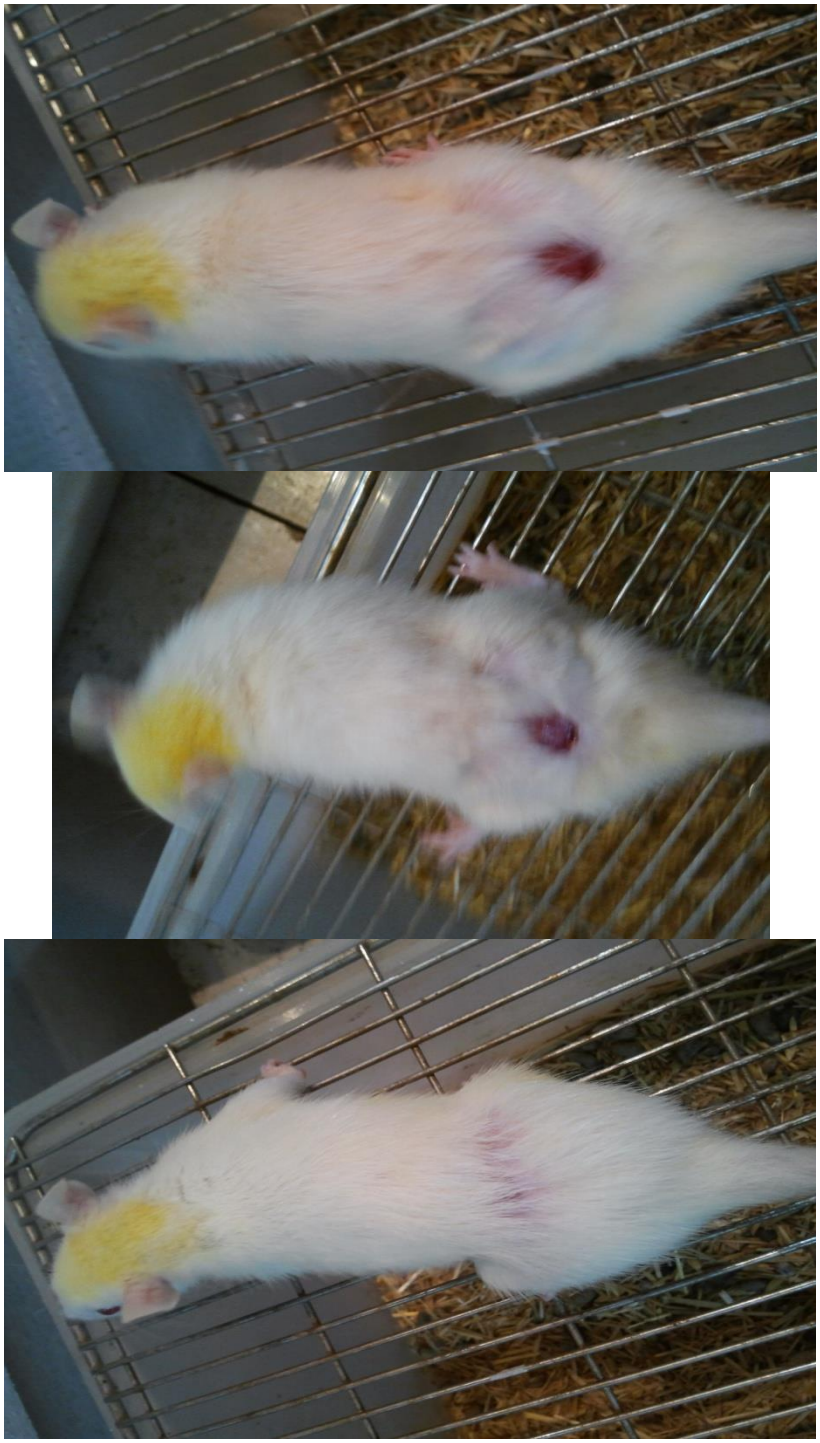

Fig 4: Effect of different treatments (as shown in table) on the rats

Table 3: showing the effect of J. regia extract on period of reepitheliazation and wound contraction

Treatment Groups	Wound Contraction by Juglans regia					
	0 days		4th day		8th day	
Head	1358.557	±7.224883	1213.933	±5.145817	706.0667	±7.521584
Tail	1387.217	±7.624627	1154.587	±5.462567	697.2867	±7.40577
Head tail	1476.137	±6.713042	1218.66	±3.880619	724.98	±8.042842
Head rt-leg	1638.257	±6.850608	1393.093	±7.300208	757.0467	±5.110551

Values represents mean ± stdev, $p<0.01$

Table 4: showing the effect of standard drug on period of reepitheliazation and wound contraction

Treatment Groups	Wound Contraction by Standard drug (Betadine)									
	0 days		4th day		8th day		12th day		16th day	
Head	173	±6.60	1540.	±7.58	1386	±6.30	1121	±7.62	902.	±5.88
	3.16	4377	157	202	.35	146	.393	5001	45	1131
Tail	1252.	±6.30	1082	±6.22	926.	±7.31	702.	±6.72	514.	±7.20
	183	032	.277	0525	8033	0604	69	0692	2267	2585

Head tail	1254.243	±7.32 6843	1080 .887	±6.91 5218	916. 7167	±6.43 4946	702. 22	±6.50 4698	501. 5467	±6.68 5509
Head rt-leg	1261 .757	±6.06 1273	109 1.55	±7.78 7548	941. 0433	±7.19 5167	711. 3433	±6.29 9145	532. 15	±7.86 6054

Values represents mean ± stdev, $p<0.01$

Histopathological examinations:

In standard and treated albino rats, excision type of wounds have shown significant healing as in fibroblasts cells, collagen fibres and new blood vesicles (Fig.4), while in control rats wounds shown incomplete healing (Fig.4). Control group has shown to slightest wound healing ability when compared to extract treated and reference ointment groups. Fibroblast cells, collagen fibres and blood vessels are prominently present in standard and extract treated group as compared to control.

Histological sections from the 16th days of healing wounds almost showed a completely finishedreepithelization process. The thickness of epidermis was similar to intact epidermis. This time period showed a typical histological picture of the proliferative phase withexpressive representation of fibroblasts and new vessels. They were mostly situated at thelayer of striated muscle in granulation tissue.

4. DISCUSSION:

The wound healing property of the plant extracts is attributed to their phytoconstiuents. These include Flavonoids, terpenoids, alkaloids, tannins and sterols etc. Flavonoids and terpenoids are known to promote the wound healing process mainly due to their astringent and antimicrobial property. Similar types of wound healing activity have been reported in *Vernonia arborea* (Manjunath et.al.2005) and *Pentas lanceolata* (Nayak et.al.2005). Tannins possess wound healing properties by virtue of their antioxidant, anti-inflammatory and antimicrobial properties (Manjunatha et. al. 2006). Sterols are reported to posses wound healing activity because of their antioxidant nature. (Manjunatha et. al. 2006).

The basic principle of optimal wound healing is to minimize tissue damage and provide an adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part.

The result of excision wound model indicates that in the first 4 days there is no significant increase in the wound contraction in all the groups as compared to the control group. The results of the 8^{th}, 12^{th} and 16^{th} day indicate that there is significant increase ($P < 0.001$) in the percentage wound contraction in the group treated with standard drug that is Betadine, 1% (w/w) methanolic extract of *Juglans regia*revealing that the extract has ability to induce cellular proliferation.

Wound healing is stepwise process, which consists of different phases such as homeostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair (Charles et al., 1995). Hence in this study, excision and incision wound models were used to evaluate the effect of methanolic extract ointment on various phases.

In excision wound, the methanolic extract showed faster healing with earlier wound contraction compared with control groups. The earlier wound contraction rate of the methanolic extract may be due to stimulation of interleukin-8, an inflammatory a-chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue (Moyer et al., 2002).The methanolic extract of*Juglans regia,*increased cellular proliferation and collagen synthesis at the wound site as evidenced by increase in total protein and total collagen contents reflected by hydroxyproline content of granulation tissues. The glycosaminoglycans are a major component of the extra cellular matrix of skin, joints, eyes and many other tissues and organs. In spite of its simple structure, it demonstrates remarkable visco-elastic and hygroscopic properties which are relevant for dermal tissue function.

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