http://dx.doi.org/10.4314/ajtcam.v12i2.11

EVALUATION OF WOUND HEALING POTENTIAL OF *RUMEX VESICARIUS* L. LEAF EXTRACT AND FRACTIONS IN RABBIT

Imran Ahmad Khan[1*], Abdul Aziz[1], Muzammal Sattar[2] , Shaukat Hussain Munawar[3], Zahid Manzoor[3], Muhammad Asif Raza[4], Ghayoor Fatima[5] . Abdul Hannan[6]

[1]Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan. [2]Departmnent of Physiology and Pharmacology, University of Agriculture, Faisal Abad, Pakistan. [3]Faculty of Medicine and Allied Medical sciences, Isra University, Islamabad, Pakistan. [4]University of Agriculture Faisal Abad (D.G. Khan campus), Pakistan. [5]Organic Plant Production & Agroecosystems Research in the Tropics and Subtropics, University of Kassel, Germany. [6]Departmnent of Plant Pathology, University of Agriculture, Faisal Abad, Pakistan.
***E-mail:** imranahmadkhandurrani@gmail.com

Abstract

Background: *Rumex vesicarius* Linn leaf extract is extensively used in folk medicine for wound cure in the sub-continent, but there is no pharmacological evidence present in support of this practice. The present study was conducted to validate the folkloric use of *Rumex vesicarius* on experimentally induced excision wounds in rabbits. Phytochemical constituents were also evaluated.
Material and Methods: Aqueous and methanol fractions of *R.vesicarius* leaf extracts were prepared and analysed for the possible presence of major phytochemical classes. A 20% w/v gel of each extract (Methanol, Aqueous) was made using Cabopol 940 in the concentration of 5%. wounds were produced experimentally in normal rabbit's dorsal region of back under ketamine anesthesia. The decrease in wound size was judged by using a scale. Povidone- Iodine treated group was taken as standard while untreated group was taken as control.
Results: Aqueous fraction (200mg/kg) showed 92.34% maximum percentage of wound healing compared to control, while, 79.71% wound healing with methanol fraction (200mg/kg). Both the extracts were found to be statistical significant and comparable to control. Furthermore, wound healing activity was found to be better than standard (Povidone-iodine) treated group which may be attributed to the faster action of the active Phytochemical constituent and their multiple mechanisms.
Conclusion: We concluded that *R.vesicarius* posses good wound healing activity and can be used as alternative medicine for wound care.

Keywords: *Rumex vesicarius* Linn , wound healing, rabbit, Povidone-iodine, excision wounds

Introduction

Rumex vesicarius L. is the most prominent member of the family Polygonaceae, locally known as "Khat palak" in south Asia. Fresh juice of *R.. vesicarius* L. leaf has been used traditionally for ally pain of toothache, for nausea, anti-venom, cooling agent, as astringent, insect bite, and as appetizer, seeds were used for dysentery (Dymoke, 1972). In Ayurvedic system of medication, it was used as stomachic (Ahirrai et al., 2012), anti tumor, analgesic, flatulence, spleen disease, high cough, asthma, laxative, bronchitis, dyspepsia, heart tr€oubles, alcoholism and biliousness (Kirtikar and Basu, 1987). In Unani system of medication, it is used as tonic, leucoderma, for scabies and diuretic (Kirtikar and Basu, 1987). In other folk medicines, it is used to eradicate piles, constipation and hiccup (Hariparasad, 2011), reptile insect, urinary affection, hepatoprotective, dysmenorrhoea, blood purifier, depurative, sedative, alkalinity, chronic catarrh, renal disorders, dyspepsia, bloody dysentery and coronary (Madhavashetty et al., 2008), vomiting (Khan et al., 2013), leucoderma, antiviral, lymphatic glyndular system disease, antidiabetic, rectal prolapsus, aphrodisiac, anti-cholesterol, impetigo and carbubuncles (Nardkarnis, 2008; Pullaiah and Ali, 1999), antioxidant (Rao et al., 2003), anthelmintics (Rao et al., 2011), stomach ache (Panjdurji et al., 2009), cancer and inflammation (Aggarwal et al., 2006), spasmogenic and spasmolytic (Khan et al., 2014), diuretic (Rao et al., 2011), anti-fungal (Amira et al., 2011), and antipyretic (Khan et al., 2014). This study reports the wound healing activity of leaf extract of *R. vesicarius* Linn and its fractions.

Materials and Methods

Drugs and Chemicals

All the drugs used in this study were of pharmaceutical grade. Povidone-Iodine Ointment (5% w/w) was purchased from Brooks pharmaceuticals pvt. Ltd. Pakistan. All other reagents and chemicals used in the study were of analytical grade.

Plant Material

Indigenous medicinal plant *Rumex vesicarius* L. is known by the local name of "Khat palak" The plant were collected from the Mondka Shahjamal area of District Muzaffar garh, Pakistan. The plant material was authenticated by expert taxonomist, Professor Dr. A. Dasti Department of Pure and Applied Biology, B. Z. University, Multan, Pakistan (voucher NO. F.P.ST-215).

Preparation of extracts

The plant material was made free from foreign adulterants and vegetative debris by hand picking and leaves were detached from the plant, washed and shade dried. Within 8 days leaves became crispy, Special electrical herbal grinder was used to form

http://dx.doi.org/10.4314/ajtcam.v12i2.11

coarse powder. Uniform dark green powder was obtained with characteristic smell. Powdered plant material (1 kg) was subjected to maceration in 90% methanol in amber coloured glass bottle at room temperature for 8 days with occasional shaking (Aziz et al., 2013) similarly aqueous maceration was made . The soaked material was first passed through muslin cloth then through Whattman-1 Filter paper to remove the vegetative material. The obtained filtrate was evaporated on a rotary evaporator by using Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland at 37ºC under reduced pressure, and the extract obtained was stored at -4°C in air tight jars in lab refrigerator.

Gel formulation

A 20% w/v gel of each extract (Methanol, Aqueous) was made using Cabopol 940 in the concentration of 5%.

Animals

Rabbits of either sex with an average weight of 1.5 kg was purchased from the Animal market Hussain Agahi Multan, After 1 hr observation of the normal dermatological and allergic behaviour, the wound healing activity was determined. Rabbits were kept under standard laboratory conditions at 27^0C room temperature with 12hr light and dark cycles. All animal experiments were performed according to the rulings of the "Animal Ethical Committee" of Baha-uddin zakariya university, Multan, Pakistan.

Acute dermal toxicity evaluation

The acute dermal toxicity was evaluated by fixed dose method on Sprague Dawley rats in accordance with guidelines of OECD (Organization for Economic Co-operation and Development). Extracts were applied topically on rats at a dose of 1000-2000 mg/kg.

Grouping of Animals

Animals were divided into 4 groups, each group containing 3 rabbits as follows:
Group 1: Negative Control (Untreated)
Group 2: Treated with Standard Povidone-Iodine Ointment (5% w/w)
Group 3: Treated with methanolic extract (200mg/kg)
Group 4: Treated with aqueous extract (200mg/kg)

Wound healing activity

Rabbits were anesthetized with Katamine before and during the creation of wounds as mentioned by Morton and Malone (1972). Back surface hairs of rabbits were shaved. The scalpel was used to cut the skin of rabbits (full thickness) to make a circular wound of 78sqmm on the dorsal thoracic region. The whole wound was left uncovered. The wound closure percentage was measured on day 1st, 3rd, 6th and 9th post wounding days. A transparent sheet was used to mark the area of the wound. The recovery of the wound areas was calculated in mm^2 by the help of graph paper. It was considered as the initial wound healing area. Topically treatment was done in all cases once daily at 10 AM. On the 9^{th} day, by using following formula, the percentage protection was calculated.

$$Percentage\ wound\ Closure = \frac{Initial\ area\ of\ wound\ at\ nth\ day\ area\ of\ wound}{Initial\ area\ of\ wound} \times 100$$

Phytochemical analysis

The crude plant extracts (Aqueous and methanolic) were initially screened qualitatively with different organic solvents and reagents to detect the presence of some phytochemical classes (Aziz et al., 2013).

Statistical analysis

Value for wound healing potential was shown as mean S.E.M. The unpaired Student's *t*-test was used to calculate statistical significance of the difference. p values of < 0.05 were considered significant and < 0.01 were highly significant.

Results

Preliminary phytochemical analysis

Preliminary phytochemical screening detected presence of tannins, phenols, saponins, anthraquinones and coumarins as constituents of the crude methanolic extract of *Rumex vesicarius* (Rv.Cr) while it tested negative for the presence of alkaloid (Table 1)

Wound healing activity

In wound healing activity of *R. vesicarius* L. showed complete wound closure with methanol fraction treated group on 9^{th} day while on 8^{th} day with aqueous fraction treated group (table 2; figure 1). This study on excision wound model expressed that all the four groups showed decreased wound area day by day. However 15.31% wound healing was observed on 9th post wounding day in group-1 animals (which could be due to self immunity of the rabbits or food containing Carrot and Spanish) where as group-2 treated animals expressed 91.01% healing. While methanolic extract showed 79.71% healing while, aqueous extract showed 92.31%

http://dx.doi.org/10.4314/ajtcam.v12i2.11

maximum percentage of healing (Table 2). Both the extracts of *Rumex vesicarius* L. are found to be extremely statistical significant with respect to control. Figure 1 clearly showed the wound healing effect of different extracts of *Rumex vesicarius L.* on rabbits.

Table 1: Pytochemical analysis of methanol leaf extract of *R. vesicarius*

Sr.No	Test	Aqueous Rv.Cr	Methanolic Rv.Cr
1	Alkaloid	+	-
2	Saponins	++	+
3	Tannins	+	+
4	Anthraquinones	+	+
5	Coumarins	+	+
6	Phenols	+	+
7	Flavanoid	+	++

Table 2: Effect of topical application of different fractions of *Rumex vesicarius L.* leaf extracts on excision wound model.

Groups	Days			
	Day 1	Day 3	Day 6	Day 9
Group 1 (Control)	80.57±12.5 (0%)	80.49±12.5 (0%)	76.29±11.3 (5.12%)	68.50±10.6 (15.31%)
Group 2 Povidone-Iodine	80.66±11.2 (0%)	38.50±7.3** (51.05%)	23.76±9.3*** (69.79%)	±2.7*** (91.01%)
Group 3 MeRv.Cr	79.32±12.8 (0%)	46.19±8.4* (41.77%)	28.97±10.5*** (64.45%)	16.19±3.1*** (79.71%)
Group 4 AqRv.Cr	78.57±12.5 (0%)	48.19±6.8* (38.43%)	26.28±6.6*** (66.3%)	5.97±1.5*** (92.34%)

All expressed as mean and standard error mean (S.E.M). Mean in columns with different letters were significantly different (ns = not significant, *$P < 0.05$ **$P < 0.01$)

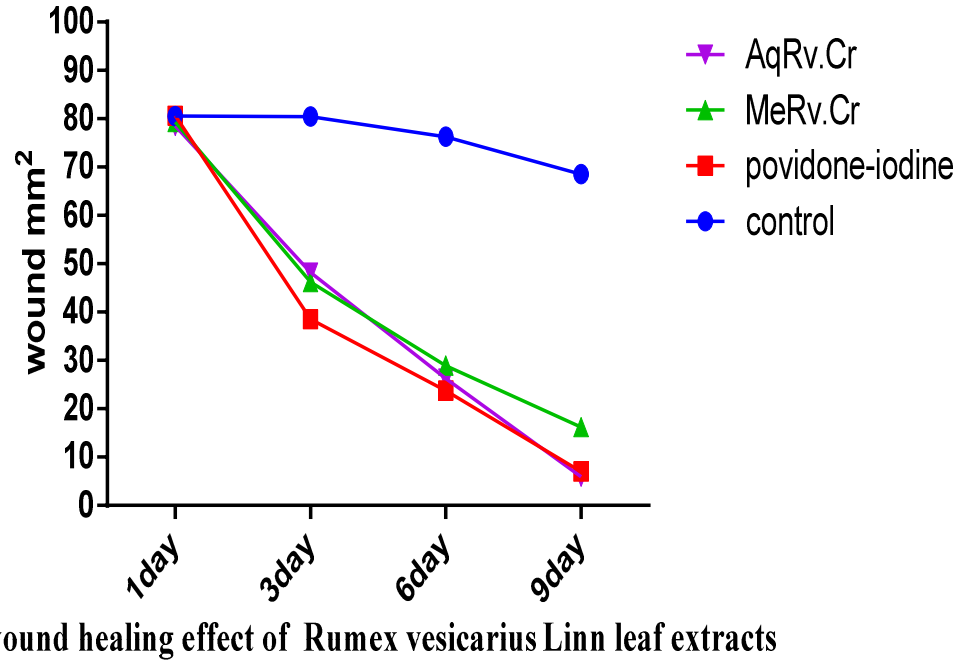

Figure 1: Shows the wound area (mm^2) of different groups after 9^{th} post wounding day

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Discussion

Various phytoconstituents and activities of the plants extracts have been reported to be responsible or helpful in wound healing. Mechanisms of wound healing may be involved in the stimulation of antioxidants production at wound site and provides a favourable medium for tissue healing (Habibipour et al., 2003). *Rumex vesicarius* leaf extract has shown excellent antioxidant activity (Hariprasad, 2011; Elbakry and Eman 2011). Antioxidant properties may serve to promote healing at the wound site. It has been reported that antioxidants may play a vital part in the wound healing process and may be an important contributory factor in the wound healing property (Habibipour et al., 2003; Getie et al., 2002) which play its role in wound healing.

Rumex vesicarius exert antimicrobial effects (Panduraju et al., 2009; Sahli and Abdulkhair, 2011) which may constitute a further basis for its wound healing activity. Microbiologically it is very significant as earlier researches concluded that the control of microbial infection is mandatory for better wound healing and its management (Levine, 1970; Muhammad and Muhammad, 2005). Inhibit the growth of fungi serve to accelerate wound enclosure, this constitute yet further wound healing properties of *Rumex vesicarius* (Amira et al., 2011). *Rumex vesicarius* showed anti-inflammatory effects (Aggerval et al., 2006), which helps to accelerate wound healing. Sivakumar (2011), claimed that gums, mucilages and phenolic compounds can be responsible for aforementioned activity. *Rumex vesicarius* has such phytoconstitents in abundance (Hariprasad, 2009; Alfaz, 2008). Hence these responsible may be responsible for wound healing activity. Tannins and flavonoids are known to enhance wound healing mechanism mainly due to their astringent and antimicrobial potential, which appears to be liable for contraction of wound and enhanced rate of epithelization (Tsuchiya et al., 1996). Both the phytoconstituents were detected in GC-MS analysis and Chromatographic finger prints of *Rumex vesicarius* leaf extract and its fractions. (Hariprasad et al., 2010). Glycosidal mixture has been reported to be liable for increased repair in incised wounds (Rosen et al., 1967), and also involved in stimulation of human skin fibroblast collagen (Umesh et al., 2010). *Rumex vesicarius* contains variety of glycosides as phytoconstituent (Alfaz, 2008), which supports the claim of above stated report. β sitosterol is one of the active compounds which may be liable for the epithelization activity (vogal and De-Souza, 1980), *Rumex vesicarius* contains β sitosterol and other steroids as phytocostituent (Khan et. al., 2013).

Conclusion

Wound healing potential of *R. vesicarius* L may be due to the phytoconstituents. Their cumulative effect may be responsible for quick wound healing. Aqueous extract out of the two extracts were studied had quick response due to presence of alkaloids .

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