

Dermatological evaluation of counter irritant effect of methanol leaf extract of *Rumexvesicarius* Linn. in rabbits

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Abstract

Objective: To assess the counter-irritant and anti-inflammatory activity of *Rumexvesicarius* in dermatological use.

Methods: The animal-based experimental study was conducted at the Royal Institute of Medical Sciences, Multan, Pakistan, in November 2014. Sodium lauryl sulfate, phenol, histamine and sandpaper irritation models were used. Irritation was induced by the clockwise frictional movement of fine sandpaper to the ear of rabbits and then applying sodium lauryl sulfate, histamine and phenol single topical application onto the ear of the rabbit. The counter-irritant effect was determined by calculating the mean decrease in redness and erythema with those of control and standard dexamethasone.

Results: There were 20 rabbits in the study with a mean weight of 1.50 ± 0.033 kg. *R. vesicarius* (100 and 150mg/mL) showed excellent counter-irritant effect when compared with control and standard groups. Both the doses depicted counter-irritant effect with the highest inhibition (94.42%) in sandpaper group, followed by sodium lauryl sulphate (90%), phenol (94.23%) and histamine (88.46%) irritation models respectively.

Conclusion: Methanol leaf extract of *R. vesicarius* countered the effect of irritation in experimental animals. It showed significant effect in terms of dose and counter-irritancy time.

Keywords: *Rumexvesicarius*, Rabbit's skin, SLS, Counter-irritant, Dexamethasone histamine. (JPMA 66: 49; 2016)

Introduction

Presentation of skin disturbance is known as "the generation of reversible harm of the skin taking over the use of a test substance for up to 4 hours".¹ Neurotic qualities and sign of skin aggravation, for example, erythema and oedema are appearances of dermal bothering. Disturbance is at first show by redness (erythema), vesicles, serous exudates, serous scabs (eschar) and different degrees of swelling (oedema). Over the long run, different responses may be shown, similar to little territories of scaling, hyperplasia, hyperkeratosis and alopecia. Histopathology is valuable in determining reactions. As a rule, aggravation takes place inside the initial 72 hours of observation.² *Rumexvesicarius* Linn. (Family: Polygonaceae) known as "Bladder dock" or "KhatPalak" is a yearly, glabrous herb, 15-30 cm in size, stretched from the root, with long, elliptic, applaud or elongated leaves and monoecious blossoms. *R.vesicarius* has been utilised customarily for toothache, nausea, as cooling agent, as an astringent, as

an appetizer, anti-venom, insect bite, and seeds for dysentery.³ In the ayurvedic system of medication, it was used as laxative, stomachic, analgesic, heart trouble, biliousness, anti-tumour, flatulence, dyspepsia, spleen disease, cough, asthma, bronchitis and alcoholism.⁴ In another tribe medicine, it was utilised to eradicate piles, constipation and hiccup.⁵ It has also been used in reptile insect, depurative, sedative, stomatic, hepatoprotective, leucoderma, blood purifier, diarrhoea, alkality, rectal prolapse, coronary heart diseases,⁶ anticholesterol, antiviral, lymphatic glandular system disease, chronic catarrh, carbuncles, antidiabetic, dysmenorrhoea impetigo, aphrodisiac, urinary infection, calculus^{7,8} as well as vomiting,⁹ antioxidant,¹⁰ spasmolytic and spasmogenic.¹¹

The present study was planned to evaluate the counter-irritant/ anti-inflammatory activity of *R. vesicarius* leaf extract.

Material and Method

The animal-based experimental study was conducted at the Royal Institute of Medical Sciences, Multan, Pakistan, in November 2014.

Rabbits of either gender were purchased from the pet market at Dolat Gate, Multan. All the drugs used were of pharmaceutical grade. Dexamethasone sodium was purchased from Orbit Laboratories (Pvt) Ltd. Pakistan.

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Sodium lauryl sulfate (SLS), histamine and phenol were purchased from G.M. Chemicals, Multan.

After observing the normal dermatological and allergic behaviour of the animals for an hour, the counter-irritant activity was conducted. Rabbits were kept under standard laboratory conditions. Experiment was performed in accordance with the rulings of the institutional Animal Ethical Committee.

Indigenous medicinal plant *R. vesicarius* was collected from the sandy soil of Shahjamal, District Muzaffargarh, Pakistan. The plant stuff was authenticated by the chief taxonomist at the Department of Botany, Bahauddin Zakariya University, Multan.

For extracts, the plant material was made free from foreign adulterants and vegetative debris, and the leaves were detached from the plant, washed and shade-dried. Special electrical herbal grinder was used to form coarse powder. The powdered plant material (1kg) was subjected to maceration in 70% methanol in amber-coloured glass bottle at room temperature for 8 days with occasional shaking.¹² The soaked material was passed through muslin cloth to remove the vegetative material, and the fluid obtained was filtered through Whatman-1 Filter paper. The filtrate was evaporated on a rotary evaporator (Rotavapor, BUCHI labrotechnik AG, Model 9230, Switzerland) at 37°C under reduced pressure (approximate yield was 11%). The extract obtained was stored at -4°C in airtight jar in laboratory refrigerator.

For the sandpaper irritation model, assay for the counter-irritant effect of *R. vesicarius* was performed as described in literature.¹³ Sandpaper with amercement particles were used to irritate the inner surface of a rabbit's ear in a clockwise direction for 10 minutes. Erythema, Irritation and redness were produced in an area of 2.0 cm² in diameter than 100mg/mL and 50 mg/mL, *R. vesicarius* and dexamethasone (standard) were applied to the irritated area. Distilled water-treated ear was used as a control. Ears were examined for the intensity of erythema. Three rabbit ears were used for each group while performing main assay by increasing concentration of irritants. Rabbits were examined after every minute. The numbers of ear-viewing decreased irritancy, redness and erythema were observed and recorded.

For the SLS irritation model, procedure described in literature was used.¹⁴ A well-known tensoactive agent was used as an irritant. This substance determines toxic dermatological effect. Concentration above 10% is

harmful. *R. vesicarius* (50,100 mg/mL) was applied directly on the skin immediately after the irritation was induced with SLS which is evident by the appearance of erythma, redness. Decrease in redness, erythma and counter-irritancy time was observed and recorded as in the sandpaper model.

For phenol-induced irritation model phenol 10% (volume/volume) pre-diluted with acetone were applied onto the inner surface of the rabbit.¹⁵ Distilled water-treated group was taken as a control, while dexamethasone (0.08mg/ear)-treated group was regarded standard. Other procedure remained the same as in the sandpaper irritation model.

For the histamine-Induced irritation model, histamine 2.5µl (0.1mg/µL) was injected by syringe having 29G-hypodermic needle for the induction of irritation/inflammation.¹⁶ Distilled water-treated group was taken as a control, while dexamethasone (0.08mg/ear)-treated group was regarded as the standard.

For phytochemical analysis, crude plant extract was initially screened qualitatively with different organic solvents and reagents to detect the presence of major phytochemical classes.¹⁷

Data was analysed using SPSS 17. Results were expressed as mean ± standard error of mean (SEM), whereby variables had first been tested for normality through Shapiro-Wilk test. Data was found normally distributed and difference between experimental groups was analysed using one-way analysis of variance (ANOVA) followed by Bonferroni test.

Results

There were 20 rabbits in the study with a mean weight of 1.50±0.033 kg. Preliminary phytochemical screening detected presence of phenols, anthraquinones, tannins, saponins and coumarins as constituents of the crude leaf extract of *R.vesicarius*, while alkaloid was found absent (Table-1).

Table-1: Phytochemical analysis of *R. vesicarius*(leaf) crude extracts (Rv.Cr).

Sr.No	Test	Observation	Results
1	Alkaloid	No traces	Negative
2	Saponins	1cm froth	Positive
3	Tannins	Light purple	Positive
4	Anthraquinones	Pink	Positive
5	Coumarins	Yellow fluorescence	Positive
6	Phenols	Light purple	Positive
7	Flavanoid	Light yellow colour	Positive

Table-2: Counter-irritant activity of *R.vesicarius* in sandpaper and SLS irritation model and degree of erythma \pm S.E.M# values along control.

Groups	Mean degree of Erythema \pm S. E. M	Sandpaper irritation model			p value	Mean degree of Erythema \pm S. E. M	SLS irritation model		p value
		Inhibition (%) of Erythema	Counter irritancy time	Inhibition (%) of Erythema			Counter irritancy time		
Control 100 μ l	95 \pm 1.66					90 \pm 1.66			
R. vesicarius 50 mg/ml	18 \pm 1.89	81.05% **	10min	0.049	21 \pm 3.55	76.66% **	17min	0.038	
R. vesicarius 100 mg/ml	5.3 \pm 2.12	94.42% ***	3min	0.000	09.3 \pm 4.1	90.00% ***	3min	0.008	
Dexamethasone 50 μ l	1.2 \pm 1.09	98.73% ***	1min	0.009	1 \pm 1.09	98.88% ***	1min	0.011	

#One-way ANOVA; * = Significant, ** & *** = Highly significant

SLS:Sodium lauryl sulfate

SEM: Standard error of mean.

Table-3: Counter-irritant activity of *R.vesicarius* in phenol and histamine irritation model and degree of erythema \pm S.E.M# values along control.

Groups	Mean degree of Erythema \pm S. E. M	Phenol irritation model			p value	Mean degree of Erythema \pm S. E. M	Histamine irritation model		p value
		Inhibition (%) of Erythema	Counter irritancy time	Inhibition (%) of Erythema			Counter irritancy time		
Control 100 μ l	92 \pm 1.66					98 \pm 1.86			
R. vesicarius 50 mg/ml	19 \pm 1.78	79.34% **	10min	0.034	27 \pm 3.65	77.17% **	17min	0.027	
R. vesicarius 100 mg/ml	5.3 \pm 2.12	94.23% ***	3min	0.003	11.3 \pm 4.1	88.46% ***	3min	0.010	
Dexamethasone 50 μ l	1.5 \pm 1.90	98.36% ***	1min	0.011	1.3 \pm 1.09	98.67% ***	1min	0.008	

#One-way ANOVA; * = Significant, ** & *** = Highly significant

SEM: Standard error of mean.

R. vesicarius (100 and 150mg/mL) showed excellent counter-irritant effect when compared with control and standard groups. Both the doses depicted counter-irritant effect with the highest inhibition (94.42%) in sandpaper group, followed by SLS(90%), phenol (94.23%) and histamine (88.46%) irritation models respectively (Tables-2 and 3).

Discussion

Positive results are associated with the presence of tannins and saponins in *R.vesicarius*. On local application, the tannins work as astringents, healing, anti-exudative, anti-irritative, anti-inflammatory antiseptic, anaesthetic and antioxidant. Tannins grade complexes with the protein from the superficial layers of the skin, leading to the formation of a protective layer of protein-tannins. Moreover, the tannins act as antiseptics through the precipitation of the proteins from the membrane of the microorganisms and anti-inflammatory through the inhibition of the synthesis of prostaglandins and the freeing of the plachetary activating factor (PAF).¹⁸

R.vesicarius reported for all the above described tannin-dependent activities. Saponins, which from a structural point of view are glycosides, have an antiseptic and

antimicrobial action in a non-harmful way to the neighbouring cellular tissues. The specialty literature draws attention to flavonoic derivatives develop anti-inflammatory effects (mainly by inhibiting the freeing of lysosomal enzymes and reducing the level of oxygen-reactive species),¹⁷ anti-allergic effects (by inhibiting the classical way the seric complement is activated), anti-microbial, capillary-protective and antioxidant. Cooling effect of *R.vesicarius* also contributes to its significance as a counter-irritant.³

Conclusion

R. vesicarius countered the effect of irritation in experimental animals. It showed a very significant effect in terms of dose and counter-irritancy time in comparison with control and standard dexamethasone.

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