



RESEARCH ARTICLE

PHARMACOLOGY

STUDIES ON WOUND HEALING ACTIVITY OF RED AND BLACK COLOURED SEED, WHITE COLOURED SEED EXTRACTS OF *ABRUS PRECATORIUS* L.



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ABSTRACT

In the present study deals with the wound healing activity of Red and Black coloured seed, White colour seed of *Abrus precatorius* L. (Fabaceae). The seed crude extract and methanol insoluble fractions of white form resulted in early wound healing activity in with and without infection. This wound healing activity due to the presence of gums, mucilages, tannins or phenolic compounds in the seeds. The results of the antimicrobial studies are promising and further support the effectiveness of the seed extracts and fraction in controlling the infection *in vivo*.



KEY WORDS

Abrus precatorius, Wound healing activity, Methanol, Crude, Gums, Mucilages, Tannins, Phenolic compounds.

INTRODUCTION

Abrus precatorius L. (Family: Fabaceae) is a climbing shrub, widely distributed in most districts of Tamilnadu in hedges and among bushes on open lands¹. Seeds usually scarlet with a black spot of uniformly black or pure white. Among variously coloured seed types² two common seed types namely red and black coloured seeds (red form) and the white coloured seeds (white form) were selected for this study. In *Unani*, the fruit is acrid with a bad taste; tonic to the brain and the body, aphrodisiac; harmful to old man. In *Siddha*, seeds used as eye disease; diseases of *pittam* and *kapham*, jaundice; pain, poisoning; arthritis, leucoderma, antidiabetic and poisonous bite³⁻⁵.

Tribes of Bundelkhand region in Madhya Pradesh use the seeds as purgative and expectorant with ginger boiled with milk. The seed presumed to have a very powerful toxic action on the nervous system⁶. The tribal of

Mirzapur district of Uttar Pradesh use the seed powder of the white form with water twice daily to treat arthritis⁷. Tribals of Nilgiris district, Tamilnadu, use the *A. precatorius* seed paste to treat nervous diseases⁸. Tribes of Chittoor district, Andhra Pradesh use the *A. precatorius* seeds to prevent, cure fistula, gonorrhoea and hair loss⁹. The Rural women of Shajahanpur district, Uttar Pradesh, use the seeds to get relief from delivery pain¹⁰. The Tharu tribe of Gouda and Bahariach district in Uttar Pradesh use the seed paste of Gumachi (*A. precatorius*) as poultice on a cotton pad and insert it into the vagina for abortion¹¹. Seed paste is used as a remedy for shoulder joints, paralysis, abortifacient, antiseptic, antitubercular and antidysenteric, useful in skin diseases, ulcer and inflammation and also useful for eye diseases¹².

MATERIALS AND METHODS

Plant Material

The fresh seeds of red and white forms of *A. precatorius* were collected from Maruthamali Hills of Coimbatore district and Mettur, Salem district of Tamilnadu, India and identified at the Botanical Survey of India, Coimbatore. A voucher specimens are kept (A.S. 1001 to 1008) in our Department herbarium for future reference.

Preparation of Extract

Shad dried seed powder was extracted with 50% aqueous ethanol in cold maceration method¹³ at room temperature. After filtration, the marc was extracted twice in the same conditions. Ethanol was removed under vacuum and the aqueous residue was lyophilized to dry the extract. Extracts (Crude-50% ethanolic extract) were fractionated in petroleum ether, chloroform and methanol. The crude (50% ethanolic extract of red form and white form) and methanol soluble and insoluble fractions of crude (red form and white form) were stored in desiccators for pharmacological studies.



Animals

Adult albino rats initially weighing between 180-220 gm were used for the study. Throughout the experimental period, the animals were housed in large spacious acrylic cages. The animals were provided with food and water ad libitum. They were maintained at a temperature range of 20-25°C. The experimental protocols were subjected to the securitization of the Institutional Animal Ethics Committee and cleared by the same.

Excision wound model - without infection

Adult Wister albino rats of either sex weighing 180-220 gm were divided into 8 groups of 6 animals each. The different contraction of gels, such as 0.5% w/v, 1% w/v and 2% w/v, using hydroxy propyl methyl cellulose (HPMC) as a gelling agent and water as a dispersing medium. These transparent and translucent gels were prepared by using stirring method with Remi-high speed stirrer (RPM 200) for 30 minutes¹⁴. The group 1 served as solvent control treated with simple gel. Group 2 was treated with positive control gel containing 5% w/v Povidone-iodine (Wokadine powder). Group 3 was treated with gel containing 2% w/v crude extract of red form, Group 4 was treated with gel containing 2% w/v crude extract of white form. Group 5 and group 6 was treated with gel containing 0.5% w/v methanol soluble and insoluble fraction of red form. Group 7 and group 8 was treated with gel containing 0.5% w/v methanol soluble and insoluble fraction of white form.

Animals were anaesthetised by the pentathol sodium (40 mg/kg ip). Then the rats were depilated on the back (dorsal side), and excision wound was inflicted by cutting away a 500 mm² full thickness of skin from a predetermined area. The wound was left undressed. The test compounds were administered for a period of 18 days, till the wound was completely healed. This model was used to monitor wound contraction and wound

closure time. Wound contraction was calculated as per cent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day (0,2,4,6.....18th day)¹⁵.

Excision wound model - with infection

The methodology used for wound formation was the same as that followed in excision wound model of without infection. It was infected with a loopful of inoculum of mixed microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans* (Ca6) and *Cryptococcus neoformans*. The mixed microorganisms were prepared by mixing 1ml of each from 10⁵ cfu/ml of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Candida albicans* (Ca6) and *Cryptococcus neoformans* cultures.

Forty-eight hours were left for infection to set in and then the treatment was started in the different groups, the test compounds were administered for a period of 18 days, till the wound completely healed. This model was used to monitor wound contraction and wound closure time. Wound contraction was calculated as per cent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on graph paper every alternate day (0,2,4,6.....18th day).

RESULTS AND DISCUSSION

The wound healing activity (without infection) was studied using 2% w/v gel prepared from the crude extract and 0.5% w/v gel prepared from the fraction of *A. precatorius* was studied on experimental wounds in albino rats. The results from the red form showed that there was significant wound healing activity

over a period of 18 days (Table 1 and 2). The percentage of wound contraction was 52.10% at the end of 6th day in the positive control applied wound, but the test gel applied (red form and white form) showed 63.86% and 65.74% Maximum healing was observed on the 8th day in the positive control but test gel treated groups significant healing was observed on the 10th day ($P < 0.01$) at 5% level by DMRT. After this period

there was no significant change in the wound area (percentage wound contraction and was found very less). Complete wound contraction (nearly 100%) was observed on 16th day with the test gel applied groups but the positive control treated even on 18th day showed the percentage contraction of only 97.17.

Table 1
The wound healing activity (without infection) of crude and fractions of red and white forms of *A. precatarius* in Wister albino rats

Past-Wounding Days	Wound area mm ² ± SE									
	Simple gel	+ control	Drug A	- Drug B	- Drug C	- Drug D	- Drug E	- Drug F	- Drug G	- Drug H
0	337.5 a ± 5.05	318.8 ab ± 17.10	280.0 a ± 25.40	278.2 c ± 18.81	306.0 b ± 7.27	305.0 b ± 9.78	307.17 b ± 4.83	303.0 b ± 4.94		
2	319.5 a ± 3.74	262.3 b ± 13.50**	234.0 c ± 17.80***	236.8 c ± 16.10***	265.00 b ± 8.34***	272.33 b ± 9.28***	258.17 b ± 4.11***	251.83 bc ± 4.29***		
4	293.7 a ± 7.89	204.3 c ± 9.70***	181.8 d ± 13.10***	182.8 d ± 8.60***	216.17 bc ± 15.95***	235.83 b ± 8.39***	211.17 c ± 3.42***	199.50 cd ± 3.31***		
6	237.5 a ± 10.15	147.7 d ± 8.9***	101.2 e ± 11.7***	95.3 e ± 4.9***	176.67 bc ± 5.49***	195.83 b ± 6.82**	159.33 cd ± 2.42**	145.17 d ± 2.52***		
8	206.1 a ± 14.22	76.0 e ± 3.30***	63.7 e ± 10.20***	57.3 e ± 4.20***	130.83 c ± 3.99***	165.33 b ± 5.75**	116.0 cd ± 1.86***	105.50 d ± 1.88***		
10	181.0 a ± 8.45	51.3 d ± 4.1***	25.2 e ± 4.1***	22.2 e ± 2.27***	97.0 c ± 2.93***	128.50 b ± 4.58***	68.50 d ± 2.63***	63.17 d ± 1.08***		
12	155.7 a ± 6.12	37.2 d ± 3.8***	14.0 ef ± 2.10***	8.3 f ± 1.20***	76.17 c ± 1.17***	100.67 b ± 3.45***	33.50 de ± 0.67***	30.17 de ± 0.60***		
14	142.3 a ± 5.71	29.3 cd ± 3.45***	5.5 e ± 0.99***	2.6 e ± 0.42***	36.00 c ± 1.13***	82.66 b ± 2.85***	11.83 de ± 0.31***	2.33 e ± 0.33***		
16	115.5 a ± 7.47	14.8 c ± 1.8***	1.5 c ± 0.43***	0.0 c ± 0.0***	11.67 c ± 0.42***	42.67 b ± 1.49***	2.5 c ± 0.22***	0.0 c ± 0.0***		
18	73.0 a ± 8.12	9.0 b ± 1.30***	0.0 b ± 0.0***	0.0 b ± 0.0***	2.33 b ± 0.21***	7.83 b ± 0.31***	0.0 b ± 0.0***	0.0 b ± 0.0***		

In a row, means followed by a common letter are not significantly different at the 5% level

by DMRT ; P values vs. respective control by Student's t - test * P < 0.05, ** P < 0.01, *** P < 0.001;



No. of animal : 06/group ; Average body weight :180-220 gms ; Route of administration : Local (gel applied using cotton swab covering the wound) ; Solvent control : Gel (without any drug) ; Positive control:5% w/v Povidone-Iodine (Wokadine

powder); Drug – A and B: 2% w/v gel contains crude extract of red and white forms; Drug – C and D : 0.5% w/v gel contains methanol soluble fraction of both forms; Drug–E and F : 0.5% w/v gel contains methanol insoluble fraction of both forms.

Table 2

Data showing percentage of wound contraction in the wound healing activity (without infection) of crude and its fractions of red and white forms of *A. precatorius* in Wister albino rats

Past Woundin g Days	Percentage of wound contraction							
	Controls		Crude		Methanol soluble fractions		Methanol insoluble fractions	
	Solvent control	Positive control	Red form	White form	Red form	White form	Red form	White form
2	5.50	17.72	16.43	14.88	13.39	10.72	15.95	16.89
4	12.97	35.91	35.07	34.29	29.39	22.69	31.25	34.16
6	29.62	52.10	63.86	65.71	42.26	35.80	48.15	58.09
8	38.93	76.16	77.25	79.40	57.25	45.80	62.24	65.18
10	46.31	83.90	91.00	92.03	68.30	57.87	77.70	79.15
12	53.86	88.33	95.00	97.02	75.11	66.98	89.09	90.04
14	57.83	90.79	98.04	99.06	88.24	72.88	96.16	99.23
16	65.77	95.35	99.46	100	96.19	86.00	99.19	100
18	78.37	97.17	100	100	99.24	97.44	100	100

The 0.5% w/v gel prepared from fractions of red form was studied on experimental wounds showed significant wound healing activity after 8th day and it was observed that the percentage wound contraction was 62.24% with the gel prepared from methanol insoluble fractions of red and 65.18% with the gel prepared from

methanol insoluble fractions of white form. The wound contraction was almost complete at the end of 14th day with both the varieties. (methanol insoluble fractions) which is also evident with the crude extracts that it produced similar activity¹⁶.

Table 3
The wound healing activity (with infection) of crude and fractions of red and white forms of *A. precatorius* seeds in *Wister albino* rats

In a row, means followed by a common letter are not significantly different at the 5% level by DMRT ; P values vs. respective control

Iodine (Wokadine powder) ; Drug – A and B: 2%w/v gel contains crude extract of red and white forms ; Drug – C and D : 0.5%w/v gel

Post-Wounding Days	Wound area mm ² ± SE							
	Simple gel	+ control	Drug – A	Drug – B	Drug – C	Drug D	Drug – E	Drug – F
0	294.16 d ± 3.87	309.5 bcd ± 6.09	315.17 abc ± 4.78	305.17cd ± 6.93	324.00 ab ± 12.09	328.17 a ± 10.18	307.17 cd ± 8.35	327.33 a ± 10.77
2	282.0 abc ± 3.80	267.17 c ± 6.29	274.17 bc ± 4.96	267.33 c ± 7.55	284.33 ab ± 10.66	297.0 a ± 9.26	266.50 c ± 7.00	284.0 ab ± 9.37
4	253.16 b ± 4.88	225.83 c ± 5.49**	230.67 c ± 4.20**	227.50 c ± 8.19*	248.17 b ± 7.87	272.17 a ± 9.04	208.00 d ± 5.62***	237.83 bc ± 77.70***
6	234.67 b ± 3.91	179.67 d ± 5.3***	150.5 ef ± 3.68***	137.5 f ± 5.47***	200.33 c ± 7.48**	258.0 a ± 8.02*	155.83 e ± 4.64	172.17 d ± 5.71***
8	217.83 a ± 8.02	156.6 b ± 5.14***	98.0 d ± 2.56***	97.33 d ± 6.63***	164.33 b ± 5.86***	205.83 a ± 6.35***	118.67 c ± 3.15***	133.33 c ± 4.51***
10	198.33 a ± 3.67	108.16 c ± 7.40***	70.17 e ± 2.47***	62.0 e ± 3.79***	142.00 b ± 5.20***	156.33 b ± 4.92***	94.50 cd ± 6.06***	90.33 d ± 2.89***
12	162.5 a ± 3.67	85.67 c ± 5.20***	49.33 d ± 3.15***	41.33 d ± 4.57***	116.17 b ± 4.42***	107.00 b ± 3.35***	57.17 d ± 1.54***	52.57 d ± 1.99***
14	140.67 a ± 2.21	65.83 b ± 4.90***	28.0 c ± 2.62***	24.5 c ± 2.88***	67.83 b ± 2.44***	69.83 b ± 2.12***	23.17 c ± 0.70***	22.0 c ± 0.77***
16	118.17 a ± 2.81	49.17 b ± 4.63***	17.0 c ± 2.39***	10.17 c ± 2.27***	44.83 b ± 4.40***	41.0 b ± 87.51***	11.50 c ± 0.43***	2.33 c ± 0.21***
18	104.5 a ± 2.81	40.0 b ± 4.38***	8.83 d ± 1.49***	2.5 d ± 1.06***	15.83 cd ± 0.65***	25.67 bc ± 1.01***	2.50 d ± 0.22***	0.0 d ± 0.0***

by Student's t - test * P < 0.05, ** P < 0.01, *** P < 0.001; No. of animal : 06 / group ; Average body weight : 180-220 gms ; Route of administration : Local (gel applied using cotton swab covering the wound) ; Microorganisms used: One loop full of mixture of bacteria and Fungi (10⁵ cfu/ml) ; Solvent control : Gel (without any drug) ; Positive control : 5%w/v Povidone-

contains methanol soluble fraction of both forms; Drug – E and F : 0.5%w/v gel contains methanol insoluble fraction of both forms

The wound healing activity (with infection) was studied using 2% w/v gel prepared from the crude extract and 0.5% w/v gel prepared from the fraction of *A. precatorius*

was studied on experimental wounds in albino rats. The results from the red form showed that

there was significant wound healing activity over a period of 18 days (Table 3 and 4).

Table 4
Data showing percentage of wound contraction in wound healing activity (with infection) of crude and its fractions of red and white forms of *A. precatorius* seeds in *Wister* albino rats.

Past wounding Days	Percentage of wound contraction							
	Controls		Crude		Methanol soluble fractions		Methanol insoluble fractions	
	Solvent control	Positive control	Red form	White form	Red form	White form	Red form	White form
2	4.13	13.68	13.0	12.39	12.33	09.49	13.14	13.24
4	13.94	27.03	26.81	25.45	23.48	17.06	32.29	27.34
6	20.22	41.95	52.25	54.94	38.33	21.38	49.17	47.40
8	25.95	49.38	68.91	68.11	49.33	37.28	61.37	59.27
10	32.58	65.05	77.74	79.68	56.21	52.36	69.24	72.40
12	44.76	72.32	84.35	86.46	64.18	67.39	81.38	83.91
14	52.18	78.73	91.16	91.97	79.08	78.72	92.45	93.28
16	59.83	84.11	94.61	96.67	86.18	87.51	96.26	99.29
18	64.48	87.08	97.18	99.18	95.12	92.18	99.19	100

The percentage of wound contraction was 41.95% at the end of 6th day in the positive control applied wound, but the test gel applied red form and white form showed 52.25% and 54.94% Maximum healing was observed on the 14th day in positive control but in test gel treated groups a significant ($P < 0.01$) activity was observed on the 10th day (5% level by DMRT).

After this day there was no significant change in the wound area (percentage wound contraction was found very less). Maximum wound contraction (97.99%) was observed on 16th day with the test gel applied groups but the positive control showed even on 18th day the percentage contraction of only 87.08 percent.



The results showed a significant ($P > 0.01$) activity after 10th day and it was maximum in with methanol insoluble fraction of both varieties (69.24%, 72.40%) and on 16th day it was found that the percentage wound contraction was nearly 100 percent. The difference in the efficiency of the gel observed between both experimental excision wound models revealed that the infection delayed the healing of wound for nearly two days and in the form of crude extract and the methanol insoluble fraction of both varieties showed parallel protection, evidences that the chemical constituents present were responsible for wound healing may be present in this fraction (methanol insoluble)¹⁶. Healing is the physiological process and does not normally require much help but still wounds cause discomfort and are prone for infection and other complications. Further, some diseases like diabetes, immunocompromised conditions, ischaemia and conditions like malnourishment, ageing, local infection, local tissue damage due to burn or gun shot wounds lead to delay in healing¹⁷.

The wound healing activity of 2% gel prepared from the 50% ethanolic extract of *A. precatorius* was significant with red form and white form on the 10th day. The wound contraction was completed earlier in the formulation containing 2% w/v of extract on 16th day, but in the positive control it was on 18th day. This clearly evidenced the presence of chemical principles in the extracts responsible for wound healing. In phytochemical analysis apart from triterpenoids and flavanoids, the presence of tannins and phenolic compounds, steroids and sterols, gums and mucilages may also be responsible for faster healing. The wound healing activity of the 0.5% w/v of gel prepared from the methanol insoluble fractions of both forms showed significant wound healing after 8th day, and complete wound contraction was observed on 14th day, shows that the fractions

were more potent in enhancing the healing faster than crude extracts, that is, it requires lesser number of days for better healing. Also the presence of gums, mucilages, tannins and phenolic compounds may be responsible for the activity. The wound healing process involves several major stages, including coagulation, inflammation, formation of granulation tissue and matrix formation and remodeling. Each of them involves very complex biological processes. During the formation of new tissue, endothelial cells proliferate and form new blood vessels¹⁸.

Neovascularization and the inflammatory response in the test wounds indicate the entire process of inflammation results in the stimulation of fibroblasts in synthesizing collagen¹⁹. Wound healing is a complex process consisting of integrated series of cellular physiological and biochemical events leading to re-establishment of structural integrity, functional restoration and regain of strength of injured tissue²⁰. The wound healing activity of the gel formulation containing 2% w/w of crude 50% ethanolic extract of *A. precatorius* showed significant activity with the infected wound model on the 6th day, which is comparatively more than the positive control. Maximum healing was observed with the formulation gel on 10th day itself, but the positive control required 14 days for the same effect, for the healing effect of the extracts even in infected conditions. But the maximum wound contraction within the formulation gel is about 97.99% on 16th day and with the positive control it was only 87.08% on 18th day.

The wound healing activity of 0.5% gel prepared from the fractions of 50% ethanol extract *A. precatorius* was studied in experimental wounds (with infection) in albino rats. The results showed significant activity with the infected wound model on the 10th day with the methanol insoluble fraction of both the forms, and it was 100% on 16th day, showing



that the fractions were more potent even in the infected condition and that they are very much effective. The antimicrobial studies results evidences that the zone of inhibition and MIC with the extracts and fractions were promising, which further support the effectiveness of extracts and fraction in controlling the infection *in vivo*. The delay in the healing due to infection for nearly two days was parallel in both the crude extract containing formulation and the fraction containing formulation, but complete healing was achieved in the fractions. Finding in *in vitro* experiments with the pathogenic microorganisms correlated with the findings of the *in vivo* experiments. Thaker and Anjaria²¹ reported the infected untreated wounds showed much delayed and incomplete healing. The synthesis of lysyloxidase an enzyme that promotes collagen maturation, which determines the breaking-strength²². Granulation depends on fibroblast proliferation, capillary bud formation and collagen laying, while the breaking-strength is determined by cross-linking and maturation of collagen²².

The process of wound contraction and epithelization is separate and independent. The activity of fibroblasts is responsible for wound contraction and involves movement of the entire dermis. Epithelization involves the migration and proliferation of cells. Stabilization of lysosomal membranes are known, inhibit cellular migration and proliferation and inhibit of fibroblast contraction which are responsible for their antihealing effects. Lipid peroxidation is an important process in several types of injuries like burns, infected wounds, skin ulcers etc. Hence, a

drug that inhibits lipids peroxidation is believed to increase the viability of cells by improving the circulation, preventing cell damage, promoting DNA synthesis¹⁵. The rate of wound contraction, period of epithelialization, skin breaking strength, granulation tissue breaking strength, dry granulation tissue weight, hydroxyproline estimation and histopathology of granulation tissue²³. The increased rate of wound contraction and decrease in period of epithelialization in the animals treated with both the extracts may be attributed to their broad spectrum antibacterial activity²⁴. Granulation, collagenation, collage maturation and scar maturation are some of many phases of wound healing which concurrently, but independent of each other²⁵.

The present findings with the extract and fraction were correlated with the earlier report on wound healing, that there is a delay in the wound healing with infection model, and healing was faster in the fraction containing formulation and wound contraction was complete. The biochemical support like lysyl oxidase, collagen synthesis and tensile strength (in incision model) further will evidence the pathways of wound healing activity. In future the histopathological observation of wound on various days further evidences the epithelization and proliferation of cells. Also and the 'lipid peroxidation' another important factor to consider, inhibition of lipid peroxidation leads to increase in the viability of cells, improving circulation, preventing cell damage and promoting DNA synthesis.

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