EVALUATION OF WOUND HEALING ACTIVITY OF RED AND WHITE SEED VARIETIES OF *ABRUS PRECATORIUS* LINN. EXTRACTS ON RATS

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Summary

Abrus precatorius (Fam: Leguminosae) is a traditional folklore medicinal plant used primarly for the treatment of wound,skin disease and ,cancer. The present study was undertaken to explore the wound healing potential of both red and white seed extracts of *Abrus precatorius* for the treatment of dermal wounds in rats. Both the extract was screened for its influence on collagenation, wound contraction, period of epithelialization and hydroxyproline content in three wound models incision , excision, and dead space wounds followed by histopathological study. Besides, its influence on oxidative stress (levels of SOD,Catalase,GSH and TBARS) was also determined in 10 day old granulation tissue. The result obtained indicated that APW accelerated pronounced wound contraction, decreased period of epithelialization, reduced wound closure time and decreased rate of granulation tissue formation and increased tensile strength and effect was equipotent as that of standard drug. Histopathological and antioxidant characters of the granulation tissue clearly suggest the therapeutic benefits of APW in the treatment of wound healing thus, justifying the local use of the plant for the treatment of skin diseases. More over APR also tends to treat wounds but at $p<0.05$ level significane compare to control group and showed delayed effect when compared with APW.

Keywords: *Abrusprecatorius*; woundhealing; antioxidants; histopathology

Running Title: Wound healing activity of Red and White Seed Extracts of *Abrus precatorius.*

Introduction

Healing of wound is an important part of the reparative process which reestablishes normal blood and lymphatic flow pattern and restores mechanical integrity of the injured system Wound healing as a prototype of tissue repair which begins the moment the tissue is injured (1) and proceeds with a complex but well-organized biological series of event that follows a predictable pattern that can be divided into overlapping phases defined by cellular population and biochemical activities a)homeostasis and inflammation, b) proliferation, c) maturation and remodeling(2). All these phases are orchestrated in a controlled manner by a variety of growth factors and cytokines (3). If resolution (repair) has not occurred after 24hrs of injury fibroblast and vascular endothelial cells begin proliferating to form a specialized type of tissue that is the hallmark of healing, called granulation tissue. Granulation tissue formation is necessary for the wound healing process (4). The formation of granulation tissue decreases with the progression of the epithelialization of the wound bed. Inadequate formation of granulation tissue or excessive formation of the components of the repair process can complicate wound healing. Chronic wounds are wounds that fail to heal despite of adequate and appropriate care. Such wounds are difficult and frustrating to manage (5) Methods currently used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which possess major drawbacks and unwanted side effects.

The plant *Abrus precatorius* Linn popularly known as Rosary pea, jequirity bean belong to the family leguminosae (Fabaceae) is found through out India in hedges and bushes in exposed areas. The seeds are deadly poisonous but it has been reported that the toxic form of abrin gets converted to mitogenic form upon long refrigerated storage. Usually seeds are of two types one is scarlet with black spot and the other variety is pure white and traditionally used against wound, leucoderma, alopecia, asthma, tubercular glands, leprosy, fever ulcer and tumor (6) (7). The objective of the pharmacology of wound heling is to study the influence of various measures in wound management programs on healing and to screen drugs that promote healing. Several materials have so far been used and are reported to affect healing differently.

However, intensive research in wound healing has not yielded, economic and efficacious pro- healing agent that could obviate the long hospitalization of patients following surgery and wound infliction. The plant *Abrus precatorius* reported earlier to have anti cancer (8) (9), anti inflammatory and analgesic activity (10). The present study is a part of a series of experiments designed to investigate the action of *Abrus precatorius* on skin wound healing in rat model.

Materials and Methods

Red and white seed variety of *A. precatorius* was collected from kollimallai hills, Salem district in Tamil Nadu during the month of April – May. Dr. V. Nandhagopalan, Plant taxonomist, National College, Tamil Nadu, India, authenticated the plants with a voucher specimen no: RHT: 12751. A voucher specimen has been stored in the Department of Pharmaceutical technology, Jadavpur University.

Preparation of Extracts

The dried, coarse powder of both seed varieties of *A. precatorius* were extracted with soxhlet extraction apparatus using ethanol. The resultant extract was concentrated using rotary vacuum evaporator. The extract of A. precatorius red seed and white seeds (APR and APW) were then freeze dried and stored in vacuum desiccators. The yield of APR and APW was found to be 23 and 16 % respectively.

Animals

Swiss Wistar rats (150-200g) of either sex were used in this study. They were maintained under controlled temperature (23 ± 2^0C) and relative humidity (40 - 60 % with standard environmental conditions of 12/12 light/dark cycle in the departmental animal house. They were housed in polypro- pylene cages with free access of food and water ad libitum. The cages were cleaned daily by changing the sawdust bedding.

The experimental protocol was approved by Institute's animal ethical committee; care and use of laboratory animals were confirmed to national guidelines.Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced.

Drug Formulation

All the doses for the test extract were fixed from the acute toxicity studies (10).Two types of drug formulations were prepared from the extracts. Topical application was made in the case of excision and incision wound model whereas, dead space wound model receives oral treatment. For topical administration, 10% w/w of extract ointments was prepared using simple ointment base BP. The drug solutions for oral administration were freshly prepared daily in distilled water and administered at 250 mg/kg bw/day orally.

Pharmacological Experiment

All the experiments were conducted in the pharmacological research laboratory between 9.00 am – 9.00 pm at a standard environmental condition (24 ± 2^0C). The drug solutions for the experiments were prepared freshly daily.

Excision Wound

Animals were anaesthetized prior to and during creation of wounds, with ether. An excision wound was inflicted in rats as described Morton and Malone (11). The dorsal fur of the animals was shaved with an electrical clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. An excision wound was inflicted by cutting away 500mm^2 full thicknesses along the marking on the depilated back 5cm away from the ears. The animals were randomly divided into four groups of six each as follows: Group I rats were treated with simple ointment base (control). Group II and Group III rats were treated with 10% w/w of APR and APW extracts ointment respectively. The extract ointments at a quantity of 0.5g were applied once daily to treat different groups of animals. Group IV rats were treated with the reference standard 0.2% w/w

nitrofurazone (NFZ) ointment. Wound closure rate was assessed by tracing the wound on every alternate days of post wounding using transparency paper and a permanent marker. The wound areas recorded were measured using graph paper. The wound was left undressed to the open environment. The day of eschar falling off, after wounding, without any residual raw wound was considered as the time until complete epithelialization.

Incision Wound

This model was employed to determine the breaking strength of incision wound in rats (12).The animals were anaesthetized with anaesthetic ether, and two paravertebral long incisions of 6cm in length were made through the skin and cutaneous muscles at the distance of about 1cm from the midline on each side of the depilated back of the rats. After the incision was made, the parted skin was kept together and stitched at 0.5 cm intervals continuously and tightly using surgical thread (No. 000) and a curved needle (No.11). All the groups were treated in the same manner as mentioned in the case of excision wound model. Simple ointment (control), extract ointments and standard drug were applied to the wound once a day from 0 to 9^{th} postwounding day. The wounds were left undressed. The sutures were removed on 7^{th} post wounding day and continued the application of the extract. The skin-breaking strength was measured on day 10 by continuous and constant water flow technique by the method of Lee (13).

Dead space Wound

Under light ether anaesthsia, in the rats, subcutaneous dead space wounds were inflicted by implanting 2.5 x 0.5 cm polypropylene tubes of two numbers in the lumbar region on dorsal side by making a pouch through a small nick in the skin (14).The animals were randomly divided into four groups of six each as follows: Group I Control rats receive 0.5ml of Normal saline. Group II and Group III rats receive APW and APR extracts (250 mg/kg bw/day) orally.

Group IV rats were treated with the reference standard indomethacin (10 mg/kg). The respective treatment was carried out from 0 day to 9^{th} postwounding day. On the 10^{th} postwounding day under light ether anaesthesia granulation tissue surrouding the tubes were carefully harvested along with the tubes. The dissected granulation tissue was employed for the determination of dry weight and was noted by drying it overnight in an oven at 60^{o}c to get constant weight. Acid hydrolysate of the dry tissue was used for the estimation of hydroxyproline using Neumann and Logan method (15). The remaining granulation tissue was used to determine the levels of Superoxide dismutase (SOD) (16), Catalase (17), reduced glutathione (GSH) (18) and thiobarbutric acid reactive species (TBARS) (19). Granulation tissues were sectioned for Histological study for all four groups of animals. The amount of collagen was quantified using Van Geison's stain.

Results

Excision wound model

APW demonstrated significant wound healing property ($P<0.001$) as that of the reference standard ($P<0.001$) group. When compared with those who received the control treatment APW showed significant reduction in wound area and increased in % of wound contraction and showed significant hastening of epithelialization at $P<0.001$ level. But the animals treated with APR showed moderate reduction in the wound area and slow rate of epithelialization and showed significane at $P<0.05$ level. The wounds treated with APW formulation shows complete healing on day 12 and day 14 for wounds treated with nitrofurazone and day 16 for APR; here again APW had a head start. Whereas control group took more than 25 days for complete healing of wounds. The study was carried out till the falling of scar leaving no raw material behind. From the results shown in the Table 1&2 it was observed that APW treatment has significant effect in hastening healing than the APR treatment when compared with control and results of APW treatment was comparable with the standard (Fig:1-6).

Incision Wound Model

The incision wound study was carried out to measure the tensile strength on day 10. The incision wounds treated with APW, APR and Standard groups tensile strength values were found to be 443.83±4.90, 323.69±8.52 and 425.78±6.64 respectively, compared with mean tensile strength of untreated group, ie, 287.45±2.34g, from the observations in Table -3 it was found that the incision wound treated with APW shows good tensile strength with $P<0.001$ significance which is comparable with standard ($P<0.001$)than the groups treated with APR extract ($P<0.05$) when compared with the control groups.

Dead space Wound Model

The results of dead space wound model were given in Table-4. The hydroxylproline estimation for the groups treated with APW,APR and standard drug shows the value 68.19±0.71 ,40.45±1.35 and 62.90±1.90 respectively, while the control group shows 31.46±1.26. From the above result it was clear the APW and standard drug treated animals had significantly greater levels of hydroxyproline than animals in APR treated groups when compare with control group ($P<0.001$).The effect produced by standard drug was found to be same as that obtained with the application of APW ointment.

The antioxidant activity such as SOD, Catalase level and reduced GSH concentration in granulation tissue of APW and APR extracts was significantly increased at $P<0.001$ and $P<0.05$ levels when compared with control. Malondialdehyde (MAD) level in granulation tissue was significantly decreased in the case APW and APR at $P<0.001$ and $P<0.05$ levels compared with controls (Table-5).

Next, we evaluate the process of wound healing histologically from histological sections of granulation tissue from all the groups. APW and standard drug treated rats showed increased and well organized band of collagen, filled with fibroblast, endothelial cells and decreased in the inflammatory cell density. Granulation tissue section obtained from control rats revealed more inflammatory cells and less collagen fiber and fibroblast (Fig:7-9).

Table 1. Effect of *Abrus precatorius* extracts on Excision wound model

Post wounding days	Wound Area in mm^2			
	Control (simple ointment)	Nitrofurazone (0.2%w/w)	APR (10%w/w)	APW (10%w/w)
0	522.16±9.04	513.16±7.89	521.66±6.76	516.67±9.74
2	521.83±8.75 (0.06)	480.83±3.43 [ab] (6.30)	502.00±5.42 (3.77)	475.66±9.24 [ab] (7.93)
4	520.00±8.88 (0.41)	375.33±4.94 [ab] (26.86)	462.16±3.21 [a] (11.40)	370.33±9.58 [ab] (28.32)
6	509.16±9.22 (2.49)	202.30±3.5 [ab] (60.57)	424.66±2.23 [a] (18.59)	188.83±10.34 [ab] (63.45)
8	475.00±8.86 (9.03)	177.20±3.42 [ab] (65.46)	390.30±2.18 [a] (25.18)	161.50±8.64 [ab] (68.74)
10	399.33±8.19 (23.52)	82.66±2.04 [ab] (83.89)	261.50±2.49 [ab] (49.87)	74.83±4.10 [ab] (85.52)
12	264.87±8.36 (49.27)	48.83±1.64 [ab] (90.48)	180.50±2.04 [a] (69.40)	23.83±1.03 [ab] (95.39)
14	227.66±8.24 (56.40)	21.66± 0.43 (95.78)	88.50±1.99 [ab] (83.61)	(100)
16	181.00±4.50 (65.34)	(100)	26.33±0.60 [ab] (94.95)	(100)
18	151.00±10.85 (71.08)	(100)	(100	(100)

All the values are expressed as mean ± SEM (n=6). Statistical significance was calculated by ANOVA followed by post hoc Dunnett's using SPSS package ([a]p<0.05; [b]p<0.001).In parenthesis,the percentage of wound contraction compared with control.

Table 2. Period of epithelialization in Excision wound

Groups	**Period of Epithilialization**
Control (simple ointment)	26.83±0.6
APW (10%w/w)	14.50 ±0.48 [ab]
APR (10%w/w)	21.16 ±1.57 [a]
Nitrofurazone (0.2%w/w)	16.33±0.67 [ab]

All the values are expressed as mean SEM ± (n=6).Statistical significane was calculated by ANOVA followed by post hoc Dunnett's using SPSS package ([a]p<0.05; [b]p<0.001).

Table 3.Effect of *Abrus precatorious* on Incision wound

Groups	**Tensile strength (g) Mean ± SE**
Control (simple ointment)	287.45±2.34
APW (10%w/w	443.83±4.90 [ab]
APR (10%w/w)	323.69±8.52 [a]
Nitrofurazone (0.2%w/w)	425.78±6.64 [ab]

All the values are expressed as mean SEM ± (n=6).Statistical significane was calculated by ANOVA followed by post hoc Dunnett's using SPSS package ([a]p<0.05; [b]p<0.001).

Table 4: Effect of *Abrus precatorius* on dry weight and hydroxyproline content of granulation tissue in dead space wound model.

Groups	Dry weight (mg) Mean ± SE	Hydroxyproline (mg/g of tissue) Mean ± SE
Control (Normal Saline)	48.38± 1.92	31.46±1.26
APW(250mg/kg)	71.13±2.26 [ab]	68..19±0.71 [ab]
APR (250mg/kg)	57.18±2.74 [a]	40.45±1.35 [a]
Indomethacin (10mg/kg)	67.25±0.47 [ab]	62.90±1.90 [ab]

All the values are expressed as mean SEM ± (n=6).Statistical significane was calculated by ANOVA followed by post hoc Dunnett's using SPSS package ([a]p<0.05; [b]p<0.001).

Table 5.Antioxidant activity of *Abrus precatorius* in dead space wound

Groups	SOD U/mg/protein	GSH µg/mg/protein	MAD level nm/mg/protein	Catalase k/s/mg/protein
Control (Normal Saline)	**27. 21±0.82**	**6.65±0.62**	**6.46±0.42**	**48.92±0.94**
APW(250mg/kg)	**46.53±1.16** [ab]	**16.27±0.88** [ab]	**2.5±0.18** [ab]	**72.23±2.12** [ab]
APR (250mg/kg)	**31.87±1.88** [a]	**8.16±0.51** [a]	**4.93±0.21**[a]	**52.62±1.43** [a]

All the values are expressed as mean SEM ± (n=6).Statistical significane was calculated by ANOVA followed by post hoc Dunnett's using SPSS package ([a]p<0.05; [b]p<0.001).

Effect of *Abrus precatorius* on excision wound model

Fig1.- 0 day wound

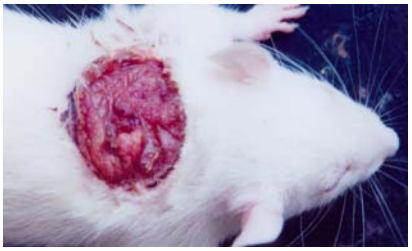

Fig.2- 6th Day wound Control

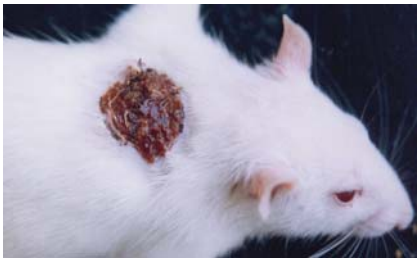

Fig.3- 6th day wound Treated APWE (10%w/w)

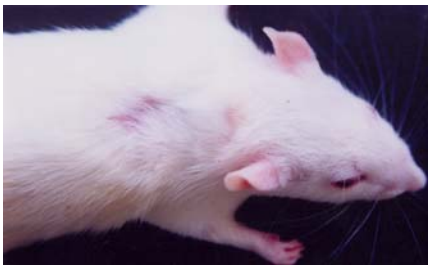

Fig.4- 14th Day Treated APR(10%w/w)

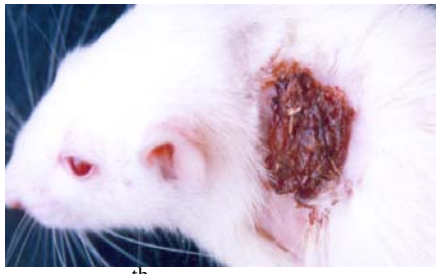

Fig.-5 14th Day Control

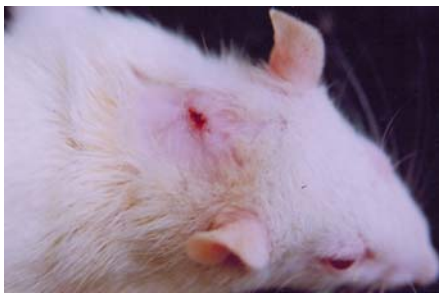

Fig.6- 16thDay Treated APR(10%w/w)

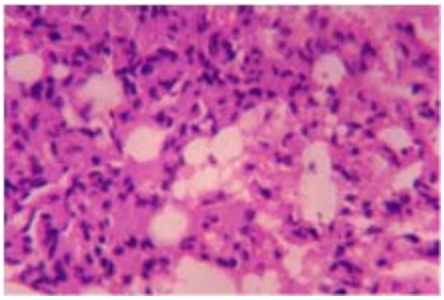

Fig. 7- Histology of granulation tissue obtained from control rats, shows more inflammatory cells and less collagen fibers

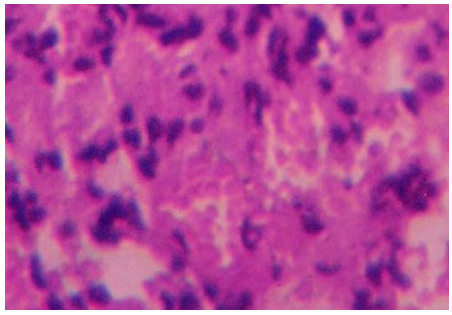

Fig. 8- Histology of granulation tissue obtained from APR (250mg/kg) rats, shows Moderate inflammatory cells and collagen fibers

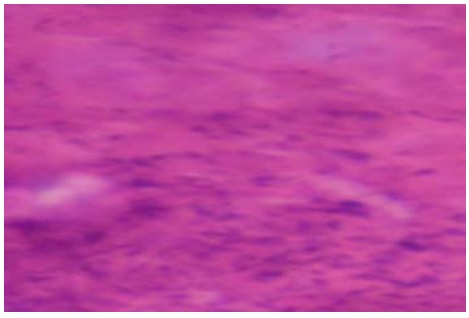

Fig. 9- Histology of granulation tissue obtained from APW (250mg/kg) rats,shows Less inflammatory cells ,well organized bands of collagen fibers and more fibroblast

Discussion

The process of wound healing is achieved by which a damaged tissue is restored as closely as possible to its normal state and the process of wound contraction is by shrinkage of area of the wound. Healing of wound mainly depends on the repairing ability of the tissue type and extent of damage and general state of the health of the tissue. Proper and timely wound healing is a vexing problem faced by all clinicians. Development of an ideal wound healing drug is still a challenge to the medical scientists. In majority of patients, normal healing establishes tissues integrity quickly and effectively. Even though at times, this healing is delayed and the ability to accelerate the wound healing becomes highly desirable (20). The ideal drug should full fill the criteria such as rapid contraction of wound leading to quick healing, reduction of wound index and appreciable gain of tensile strength (21). From result of excision wound study, it was concluded that the APW treatment showed better and faster healing as compared to the untreated group. From the initiation of the study there was a much better difference in the wound healing for APW.APW shows pronounced wound healing activity from the beginning till the end than APR treatment. We observed that the healing potential of APW was comparatively similar indicating similar potential over nitrofurazone 0.2%w/w cream for wound healing activity . The results in Table-1 indicated that out of the two extract ointments used in the experiment , APW has been found to have relatively good wound healing activity with 100% of wound closure on day 14 as compared to the standard group and it is capable to treat excision wounds like abrasives. A considerable difference in response between the two extract ointments APW and APR was noted on wound closure. Complete wound contraction took place in APW extract 2 days before that of the standard drug.

The tensile strength of a wound represents the degree of wound healing. Clinically increased wound strength is an important aspect in healing of surgical wounds. Scar weakness can lead to wound dehiscence and incision hernia. Wound healing agents usually provide a gain in tensile strength. Drugs like NSAID, steroids and antineoplastics have adversely affected healing in the pre-surgical period (13) (22) (23).

From our study we confirmed that systemic administration of APW extract can prevent such an eventuality and can be used in the healing process of surgical wounds.In this incision wound model APW showed significant (P<0.001) wound breaking strength compared to control group, the increase in tensile strength of treated wound may be due to increase in collagen concentration/unit area and stabilisation of the fibres (24).

An agent that influence the collagen turnover or maturation could be expected to promote wound healing (25)Since collagen is the principle component of any repaired tissue. Collagen is a fibrous protein component of connective tissue and provide a structural frame work to the tissue (26) (27) consisting of hydroxyproline, hydrolysin and glycin as principle constituents among which hydroxyproline is considered a special amino acid which gives strength and support.Break down of collagen liberates free hydroxyproline and its peptides. Its synthesis is stimulated by various growth factors (28) which is also known to promote the proliferation of fibroblasts and fibroblast proliferation from the granulation tissue (29). Measurement of hydroxyproline could be used as an index for collagen turnover. Hence its estimation in the granulation tissue may throw light on the maturaion and healing process (30). In the dead space wound model APW treatment shows significantly greater levels of hydroxyproline than the animals in control group (P< 0.001),where as APR shows significane at P<0.05 level when compared to control group. Further the cell proliferation can best be judged on the basis of histological study. The reactive oxygen species (ROS) are deleterious to the wound healing process due to their harmfull effects on cells and tissues (31). Overproduction of ROS result in oxidative stress thereby causing cytotoxicity and delayed wound healing. Hence, elimination of ROS could be an important part in chronic wound healing (32).Hence, antioxidants like SOD,Catalase and glutathione estimation in granulation tissue is also relevant because these antioxidants destroy the free radicals and hasten the process of wound healing (33). Estimation of free radical scavenging properties of both the extract have prooved that APW have potent anti oxidant activity than APR extract.Hence APW improve significant wound healing and protect tissues from oxidative damage than APR treatment.

From the above findings we have confirmed the wound healing effect of APW and APR in excision, incision and dead space wound models. Therefore, it may be concluded that APW shows pronounced and significant wound healing activity in both topical and systemic application as comparable with standard drug in all the three models when compared with the control group. These findings would justify, at least partially, the inclusion of this plant in the management of wound healing in folk medicine. Since the role of free radicals and antioxidants in wound healing are very clearly defined, wound healing potential of APW may be partly due to the potent antioxidant activity of the plant.

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