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Wound Healing Potential of *Abrus Precatorius* in Normal and Diabetic Rats

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ABSTRACT

The ethanolic extract of *Abrus precatorius* leaves was screened for Wound Healing potential in normal and diabetic rats using excision and incision wound model. In excision wound model, normal and alloxan induced diabetic group of animals treated with AP 5% and 10% ointment showed significant increase in wound contraction and decrease in epithelization period as compared to control. In incision wound model, normal and alloxan induced diabetic group of animals treated with AP 5% and 10% ointment showed significant increase effect on breaking strength compared with control group. Histopathological findings of AP 5% and 10% ointment also showed, less inflammatory changes, increase tissue proliferation as well as remodelling along with re-epithelization, increase collagen fiber along with low scar formation as compared to control group. Findings of the results thus revealed that the ethanolic extract of *Abrus precatorius* (AP) has showed significant effect on wound healing activity in both normal and diabetic group of rats by minimizing the undesired consequences and shortening the period of wound healing.

Keywords: *Abrus precatorius* wound healing, excision, incision.

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INTRODUCTION

Wounds are unavoidable events of life. It is accompanied with pain, the intensity of which may vary from low to extreme and a person who experienced pain is termed to be hurt and develop a wound at the place of injury. Healing of wound is basically a survival phenomenon and represents an attempt to maintain normal anatomical structure with function. When healing takes place in a direction away from its normal course, it is common to have under or over healing. Treatment is therefore aimed to fasten the recovery process of healing or minimizing the undesired consequences. Traditionally, leaves of *Abrus precatorius* belonging to family Fabaceae are being used as nerve tonic, applied on cuts, wounds and swellings and mouth ulcer. The plant is also traditionally used to treat tetanus, and to preventrabies. It possesses antimicrobial[1], antifertility[2], anti-implantation and antiovulatory [3], immunoomodulatory[4] anti-tumor[5], and anti-inflammatory activity[6]. The seeds of *Abrus precatorius* showed presence of Abrin, a flavonol glycoside[7], toxalbumin, indole derivative, anthocyanin, sterols, terpenes[8]. Arial parts showed presence of Triterpenoids saponins 1 and 2, isoflavanchinones -abruquinone B, G.[9, 10].Roots showed presence of Saponins, Isoflavanquinones- abruquinones D, E, and F; Precol, abrol, alkaloids, precasine, triterpenoids, indoles, sterols[11] and leaves showed Four triterpenoids-abrusosides A-D and saponins[12]. Monago & Alumanah, (2005) investigated that Chloroform–methanol extract of *Abrus precatorius* has shown to have antidiabetic properties in alloxan diabetes in rabbit. Pal *et al.* (2009) proved the potential *Abrus precatorius* for antioxidant activity. These characteristic antioxidant properties of *Abrus precatorius* may serve to promote healing at the wound site. It was also found that two triterpenoid saponins 1 and 2 isolated from the aerial parts of *Abrus precatorius* and their acetates derivatives, 3 and 4 have anti-inflammatory activity[6]**.** The crude extract of seeds and methanol insoluble fractions of white form of *Abrus precatorius* is having wound healing activity in Excision wound model with and without infection in rats[15]. These all may constitute for the evaluation of wound healing activity of *Abrus precatorius.* A comprehensive literature survey revealed that there are no scientific studies carried out regarding leaves of *Abrus precatorius* in wound healing effect in Normal and Diabetic rats. Hence, the present study is focused to evaluate the wound healing potential of leaves of *Abrus precatorius* using excision and incision wound model.

MATERIALS AND METHOD

Collection and Authentication of Plant material

The leaves of *Abrus precatorius* purchased from local market of Pune and authenticated by Agharkar Institute of India. Authentication number is Auth.13-266.

Preparation of Extracts:

About 2000gm of leaves of *Abrus precatorius* were dried under shed and coarsely powdered. Leaves were defatted with petroleum ether and then subjected to maceration process by using 70% ethanol for 7 days shaking occasionally. After 7 days mixture was filtered and filtrate was evaporated to dryness to give ethanolic extract of *Abrus precatorius* (AP).Ethanolic extract of *Abrus precatorius* (AP) was stored under desiccators for further pharmacological and phytochemical studies[16].

Figure 1: Abrus Precatorius

Procurement and housing of Animals:

The Wistar rats of either sex were purchased from National Toxicology Centre, Pune. They were housed in group of five under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra) and water *ad libitum*. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA (CPCSEA NO.198/99) with IAEC clearance proposal numbers [DYPIPSR/IAEC/13-14/P-02].

Preliminary Phytochemical Evaluation:

The extract obtained was subjected to chemical tests for identification of various phytoconstituents[17].

Preparation of Ointment:

An ethanolic extract of *Abrus precatorius* (AP) was used for preparation of ointment using simple ointment base. Briefly wool fat (2gm), hard paraffin (2gm), white soft paraffin (34gm), cetostearyl alcohol (2gm). Each ingredient heat and mixed gently and stirring. After that cooled and pour in to airtight container stored in refrigerator. For preparation of 10% and 5% ointment 4gm and 2gm respectively added in to melted simple base stirred slowly and gently and make homologous ointment. After that cooled and pour in to airtight container and stored in refrigerator until further use. (Table 1)

Physical evaluation of Ointment:

Preliminary physical and physicochemical evaluation of ethanolic extract of *Abrus precatorius* (AP)formulation carried out[18](Table 4).

Skin irritation test:

Three healthy young adult albino rats of either sex were fed commercial pellets diet and water ad libitum. Animals were acclimated to laboratories conditions for a period of 7 days prior to initiation of dosing. Animal room was kept at a constant temperature (19-24^{O}C). On the day before application, hairs of rats were removed from the dorsal and trunk area using a small animal clipper. On the day of dosing, but prior to application, the animals were examined for health and the skin checked for any abnormalities. No pre-existing skin irritation was observed. Two to three grams of the ointment was applied to intact area on each animal and caged. After 4 h of exposure to extract ointment, the test sites were gently cleaned from any residual substance. Individual evaluation of test dose was scored according to Draize Scoring System at approximately 1, 24, 48 and 72 h after removal of extract ointment. The degree of irritancy was obtained by calculating the primary dermal irritation index (PDII). The animals were observed for next seven days for any sign of erythmea and edema[19].

Excision wound model in normal rats:

Rats were divided into 4 groups (n=5). The back of each rat was shaved under ketamine (1 ml/kg) anesthesia and prepared for excision. Thereafter open circular wound of 300 mm^2 area was produced in each rat by excising the skin using sharp sterilized scissor. For this purpose a marker was used to mark the area to be excised. Drug treatment was applied after 24 hour of wound creation to respective group of animals every day till the wound was completely healed. The wounded animals were kept separately and were left undressed to the open environment. This model was used to monitor measurement of wound area, wound contraction and epithelisation time. The percentage of wound healing was calculated according to original wound

size i.e. 300mm^{2}for each animal of group and trace wound every 2 days i.e. 2, 4, 8, 10, 12, 14, 16, 18for final analysis of results. Falling of scar leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization. Histopathological analysis of skin was done with haematoxylin and eosin[20].

Incision wound model in normal rats:

Rats were divided into 4 groups (n=5). The back of each rat was shaved under ketamine (1 ml/kg) anaesthesia and prepared for operation. Incision wound of full thickness of skin of 3 cm long parallel to the paravertebral region was made with sterile scalpel. After complete haemostasis the wound were closed by means of interrupted sutures placed at equidistance points about 1 cm apart. After 24 hour animals of each group were treated daily with respective topical formulations. The sutures were removed on the 8thpostwounding day and the tensile strength of the wound was measured on the 10th day. For measurement of tensile strength, the anesthetized animal was secured to the table, allis forceps were firmly applied on either side of incision wound 3 mm away from wound margin on adjacent normal skin. The forceps on one side was hooked to a fixed metal rod while the other forceps was attached to a freely suspended lightweight plastic of volume 1000 ml through a string run over to a pulley. As soon as gapping of the wound occurred, addition of weights was stopped and simultaneously the weights were lifted so as to avoid opening of the entire wound. The weights required to produce gapping were noted[21].

Excision and Incision wound model in Diabetic rats:

The overnight fasted rats were made diabetic by a single I.P. injection of alloxan monohydrate (150mg/kg) dissolved in saline to overnight fasted animals. It was followed by 0.5ml of 25% dextrose after 2hours of alloxan and 5% dextrose solution ad libitium for next 24hours.After 72hours of alloxan injection, blood samples were withdrawn from rat tail vein and blood glucose levels were estimated in all animals. Animals with normal blood glucose level ≥200mg/dl (diabetic) were selected for study. Animals were divided into 4 groups (n=5). The back of each rat was shaved under ketamine (1 ml/kg) anesthesia and prepared for operation. The procedure for excision and incision described in normal rats was repeated[22].

Statistical Analysis: All result data of wound healing were expressed as mean ± SEM. The groups were evaluated using one-way ANOVA followed by Dunnett's test. The data was considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Contraction, wound closure and restoration of the functional barriers are the various phases of wound healing. Wound healing is the body natural process of regenerating dermal and epidermal dermal tissue. The healing primarily depends on the repairing ability of the tissue in addition to type and degree of damage and general health status of the tissue. Ointment is most preferable form of dosage as it is the most convenient for topical application[23]. A wound produced in excision wound model mainly heals by contraction & epithelization, the monitored parameters in this model include wound closure, time required for complete closure and epithelization. Edema, fibroblast, collagen and new blood vessels are the primary component of the granulation tissue of the wound. At the site of injury when platelet comes into the contact with exposed collagen to release the clotting factors which might have resulted in formation of fibrin clot. This fibrin clot can serve as a pro metric visional which ultimately healed the wound[24]. For the tissue repair, collagen deposition can be necessary event that might have occurred through the released of fibroblast which is a connective tissue[25]. The capability of collagen fibre is increased by inhibition of lipid per oxidation. It also prevents the cell damage and promotes the DNA synthesis. Angiogenesis in granulation tissue improve blood supplementation to the wound site, thus providing nutrients and oxygen essential for the healing process[26]. Wound contraction was expressed as percentage reduction of original wound size and was determined by using following formula:

$$\% \text{ Wound Contraction} = \frac{\text{Healed Area}}{\text{Total Area}} \text{X } 100$$

To apply this equation, at 2-day intervals, the wound margins were traced and measured to calculate the non-healed area which was then subtracted from the original wound area to obtain the healed area. The percentage of wound contraction was determined first time on the 2ndday after the application of drugs and carried out at 2-day intervals for the duration of 3 weeks. After cold maceration using 70% ethanol for 7 days, the yield obtained for leaves of *Abrus Precatorius* was 42 % w/w (AP). The ethanolic extract of *Abrus Precatorius* has shown the presence of glycosides, carbohydrates, steroids and terpenoids, flavonoid and saponins. AP 5% and 10% in ointment base formulation did not show any dermal irritation, redness, or swelling on rat's skin after topical application and it therefore was found to be safe. (Figure 2) (Table 1).

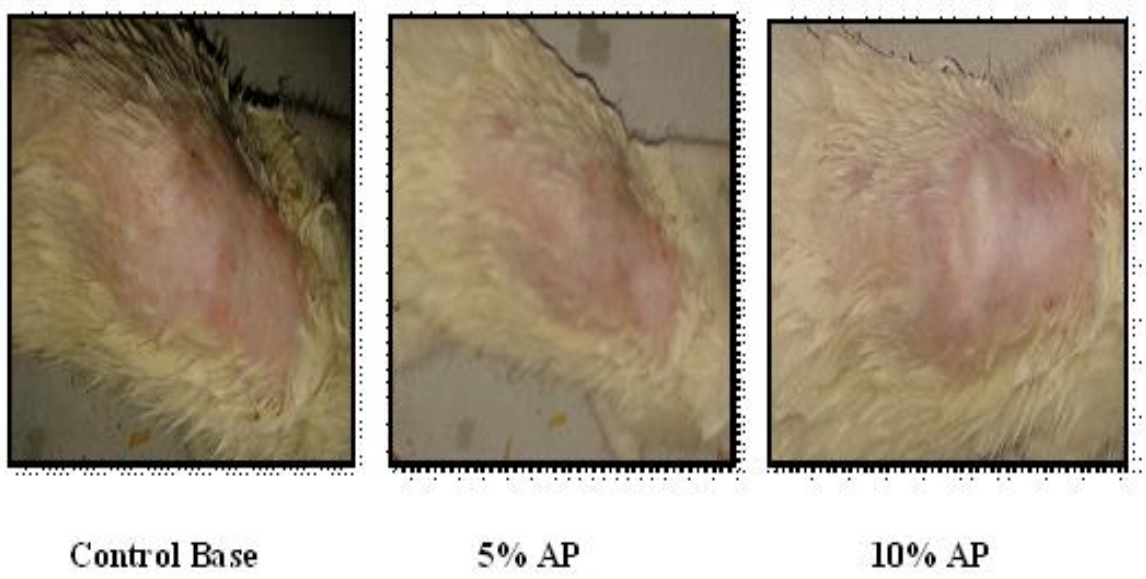


Figure 2: Evaluations of Control Ointment Base and AP 5% and 10% in Ointment Formulation for Skin Irritation

Table 1 Evaluation of AP Formulation for Physicochemical Parameters

Formulation	pH	Appearance	Colour	Spreadability (sec)	Percentage drug content	Viscosity	In vitro diffusion (drug content uniformity)
Simple base	6.7	Consistent	White	5	-	350000	-
5%AP EtOH	6.8	Consistent	Greenish	6	87.91	277000	83
10%APEtOH	6.9	Consistent	Greenish	7	91.87	61000	87

Excision and Incision wound model in non diabetic rat:

In excision wound model, 5% and 10% of the ethanolic extract of *Abrus Precatorius* ointment showed significant wound healing activity ($P < 0.01$) on 4^{th}, 6^{th}, 8^{th} ,10^{th}, 12^{th}, 14^{th} $16^{th}$and 48^{th}day in non diabetic rats as compared with control group. The percentage of wound contraction in animals treated with Nitrofurazone ointment (0.2%w/w), AP 5% and 10 % was increased significantly on 20^{th} day in non diabetic rats (Figure 3).

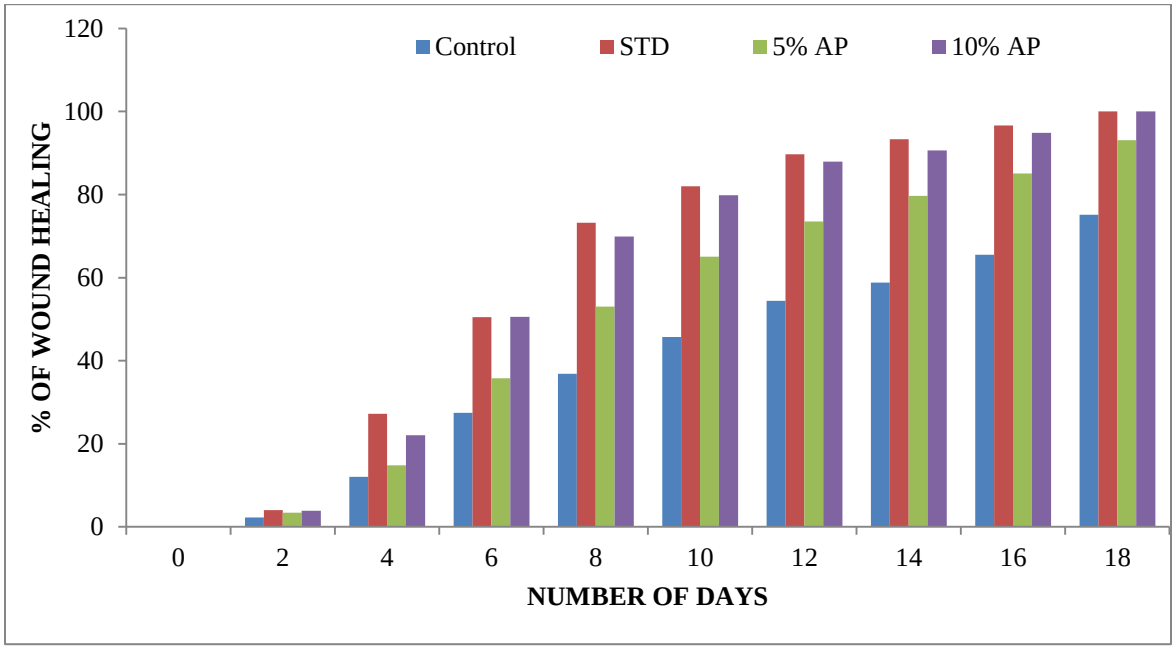


Figure 3: Percentage of Contraction Wound in Excision Wound Model in Normal Rats

In non diabetic group, epithelization period of wound in control group of animals was found to be 25.4 days. The animals treated with nitrofurazone ointment (0.2%w/w), 5% and 10% of AP ointment, there was significantly shorter epithelization period ($p<0.01$) as compared to control group of animals i.e., 18.1, 21.1 and 17. 2 days respectively. (Figure-4). Wound healing effect of AP may be due to regulation of collagen expression and an increase in tensile strength of the wound by inhibiting the elevated levels of lipid per oxidation and also increase in angiogenesis. The result of present investigation was with the coincided with the earlier work reported by evaluation of wound healing activity of red and white seed varieties of *Abrus precatorius* Linn extracts on rats[27].

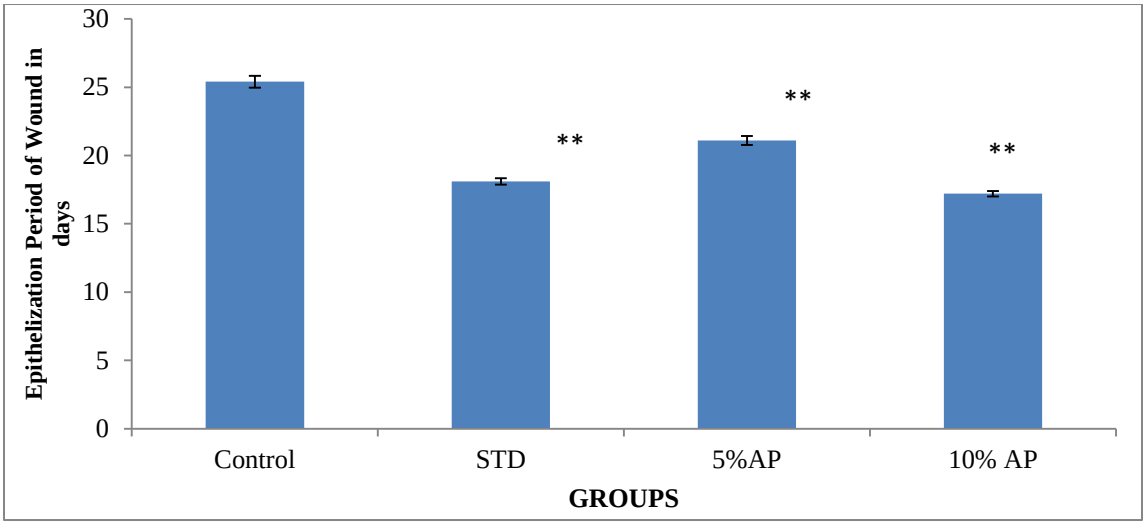


Figure 4: Epithelization Period of Wound in Excision Wound Model in non-diabetic Rats

Control = Simple ointment base (0.5 gm); **STD** = Nitrofurazone ointment (0.2%w/w); **AP 5%** = 5% w/w ethanolic extract ointment of leaves of *Abrus precatorius* in Simple ointment base; **AP 10%**=10% w/w ethanolic extract ointment of leaves of *Abrus precatorius* in Simple ointment base.

In Incision wound model, wound breaking strength of 10-day old wounds was evaluated by slow water flow technique. Wounds that are simple can be closed by sutures, tapes, or steples heal by primary intention[28]. The main mechanism of healing during primary intention is connective tissue deposition, where collagen, proteoglycans & proteins are deposited to form new extracellular matrix. In resutured incision wound model, wound breaking strength is determined which indirectly represent the collagenation phase of healing & this parameter is commonly used to assess the healing, perhaps because surgeons are specially interested & concerned with the strength of healed incision wound[24]. In Incision wound model, tensile strength was measured on 10thday. In non diabetic group of animals, treatment with nitrofurazone ointment (0.2%w/w) tensile strength (418.58 gm) was significantly ($p<0.01$) greater than that of the control group (273.34 gm) (Figure 5). The tensile strength in animals treated with AP ointment 5% (318.50 gm) and AP ointment 10% ointment (419.53gm) was significantly ($p<0.01$) greater than that of the control group.(Figure 6 & 7). The increase in the tensile strength of the wounds by *Abrus precatorius* ointment may be due to increase in collagen concentration per unit area & stabilization of fibers. The importance of cross-linking between collagen molecule & the physical weave of collagen fibres in contributing to the tensile strength is well acknowledged by Udupa (1995).

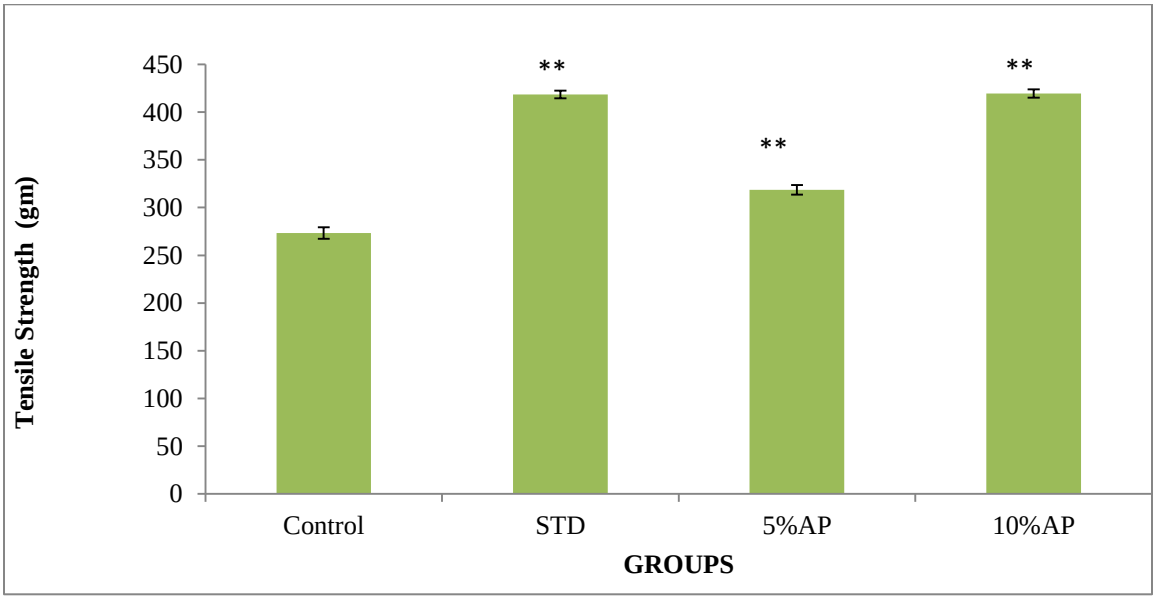


Figure 5: Tensile Strength in Incision Model of Normal Rats

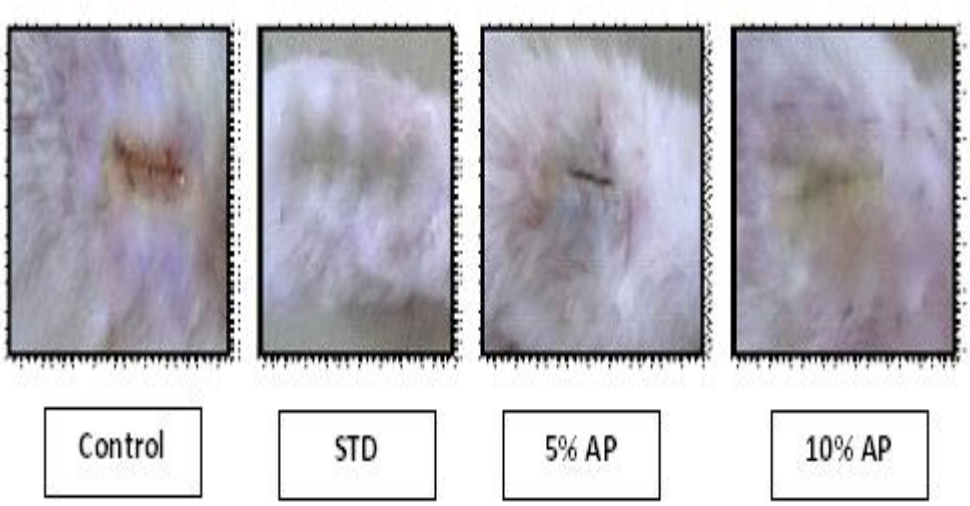
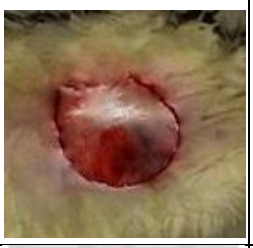
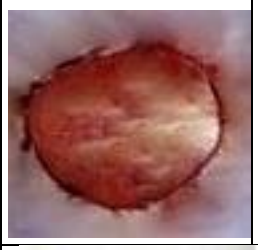
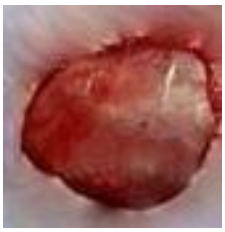
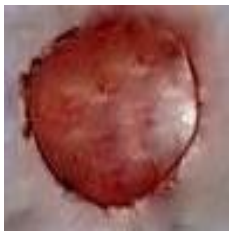
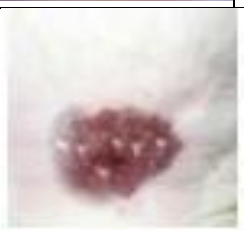
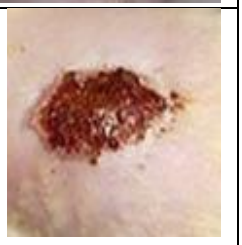
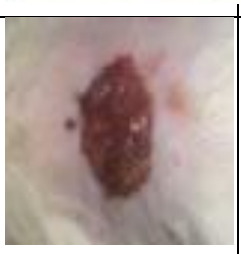
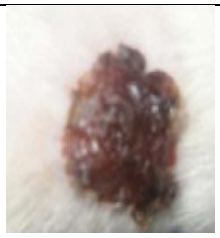
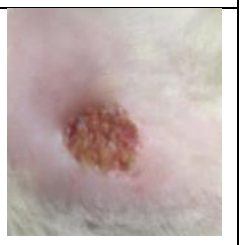
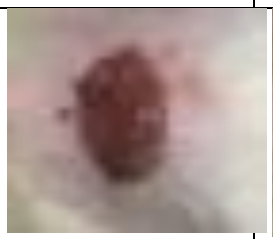
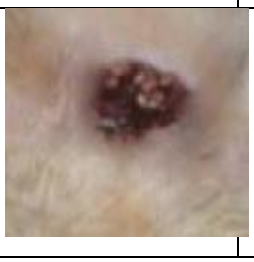
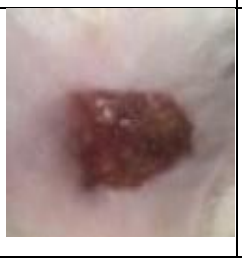
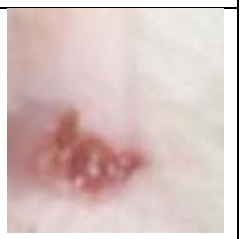


Figure 6: Tensile Strength in Incision Model of Normal Rats

DAYS	GROUPS			
	Control	STD	5% AP	10% AP
0				
4				
8				
12				

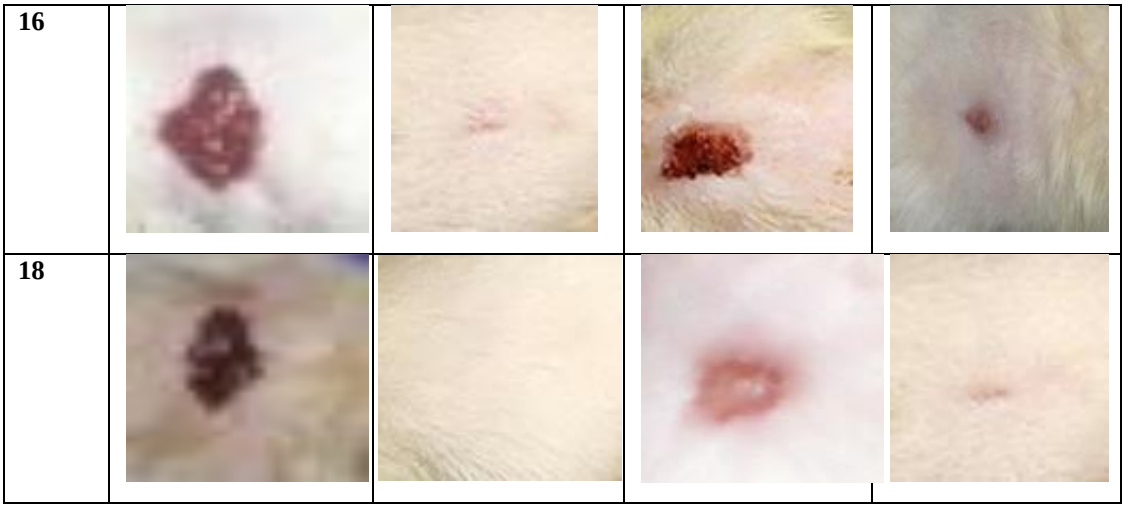


Figure 7: Process Wound Healing in Incision Model in Normal Rats

The histological evaluation showed that increased cellular infiltration from haematoxylin and eosin staining in treated cases may be due to chemotactic effect enhanced by the crude extract which might have attracted inflammatory cells towards the wound site[30]. In non-diabetic control group of animals, delay in wound healing processes along with edema and inflammation, inflammatory changes, less tissue proliferation and collagen fiber was observed. In case of animals treated with STD (Nitrofurazone ointment), 5% AP and 10% AP ointment, less inflammatory changes, increase tissue proliferation as well as remodelling along with re-epithelization, increase collagen fiber along with low scar formation was observed in non diabetic as compared with control group. (Figure 8). Increased cellular proliferation may be due to the mitogenic activity of the *Abrus precatorius*, which might have significantly contributed to healing process. Early dermal and epidermal regeneration in treated rats also confirmed that the extract had a positive effect towards cellular proliferation, granulation tissue formation and epithelization.

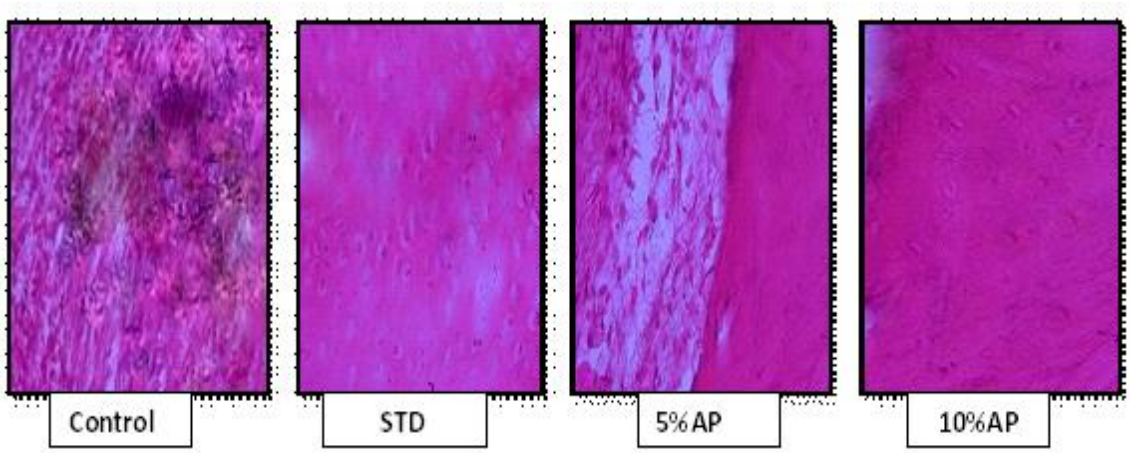


Figure 8: Histopathological Examination of Wound Membrane in Normal Rats

Excision and Incision wound model in Diabetic rats

Diabetes is the major metabolic disorder affecting many organs because of altered carbohydrate, protein and fat metabolism. Delayed wound healing is an important complication in diabetic patients. This is caused by impaired blood flow and oxygen released from increased blood sugar, decreased collagen and fibronectin from protein malnutrition, impaired local immune and cell defences, and decrease anabolic activity with decreased insulin and growth hormone. Collagen fibrin and keratin accumulate advance glycationamadroi end products with effect of binding of regulatory molecules, susceptibility to proteolysis and finally decrease the ability for protein cross linkage[31]. Alloxan is an oxidation product of uric acid. Physical appearance of alloxan is pale raddish colour. At a low dose it inhibits glucokinase activity but at high dose induce cell death. Alloxan is commonly used to produce diabetes mellitus in experimental animal due to its effect on β-cells. It destroys the β-cells of pancreas by generating excessive reactive oxygen species (ROS) such as superoxide radicals, hydroxyl and hydrogen peroxide radicals. Reactive oxygen species play important role in the pathogenesis and complication of diabetes mellitus. The action of ROS is increase amount of cytosolic calcium concentration and cause rapid destruction of cells[32]. In excision wound model, 5% and 10% of the ethanolic extract of *Abrus Precatorius* ointment in diabetic rats showed significant wound healing activity ($P < 0.01$) on 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th}, 14^{th}, 16^{th} and 48^{th}day as compared with control group. The percentage of wound contraction in animals treated with Nitrofurazone ointment (0.2%w/w), AP 5% and 10 % was increased significantly on 20^{th} day in diabetic rats (Figure 9).

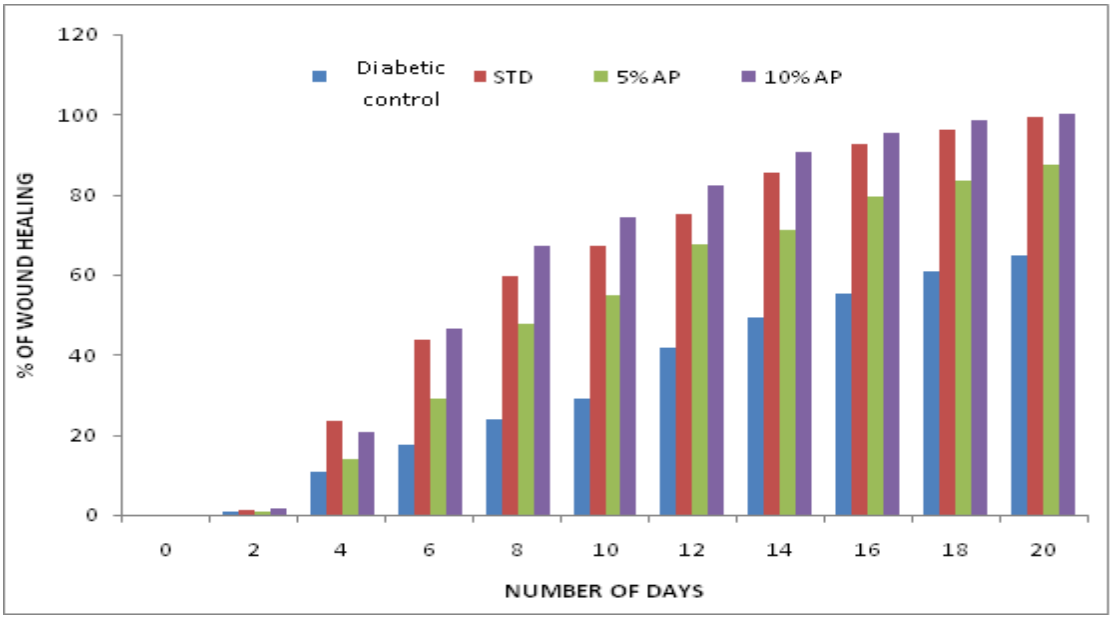


Figure 9: Percentage of Contraction Wound in Excision Wound Model in Diabetic Rats

Epithelization period of wound in diabetic control group of animals was found to be 27.4 days. In diabetic group, animals treated with nitrofurazone ointment (0.2%w/w), there was significantly shorter epithelization period ($p<0.01$) as compared to control group of animals i.e., 20.3 days respectively. In diabetic group of animals, animals treated with 5% and 10% of AP ointment, there was significantly shorter epithelization period ($p<0.01$) as compared to control group of animals i.e., 22.6 and 19.1 days respectively (Figure 10). The same results were observed by wound healing activity of ethanolic extract of *Allium sativum* on alloxan induced diabetic rats[22].

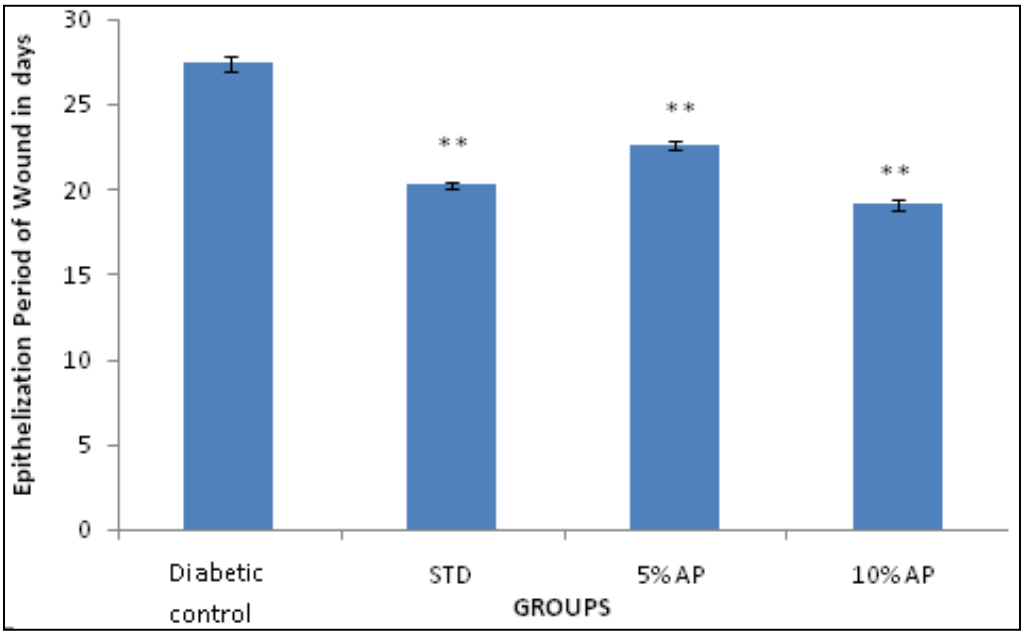


Figure 10: Epithelization Period of Wound in Excision Wound Model in Diabetic Rats

In Incision wound model, tensile strength was measured on 10th day. In diabetic group of animals, tensile strength of the animals treated with standard group treated with Nitrofurazone ointment (0.2%w/w) was significantly ($p<0.01$) greater (398.41gm) than that of the diabetic control group (222.15 gm) (Figure 11). In animals with AP 5% ointment (300.47gm) and AP 10% ointment (402.39 gm), tensile strength was significantly ($p<0.01$) greater than that of the diabetic control group (Figure 12 & 13).

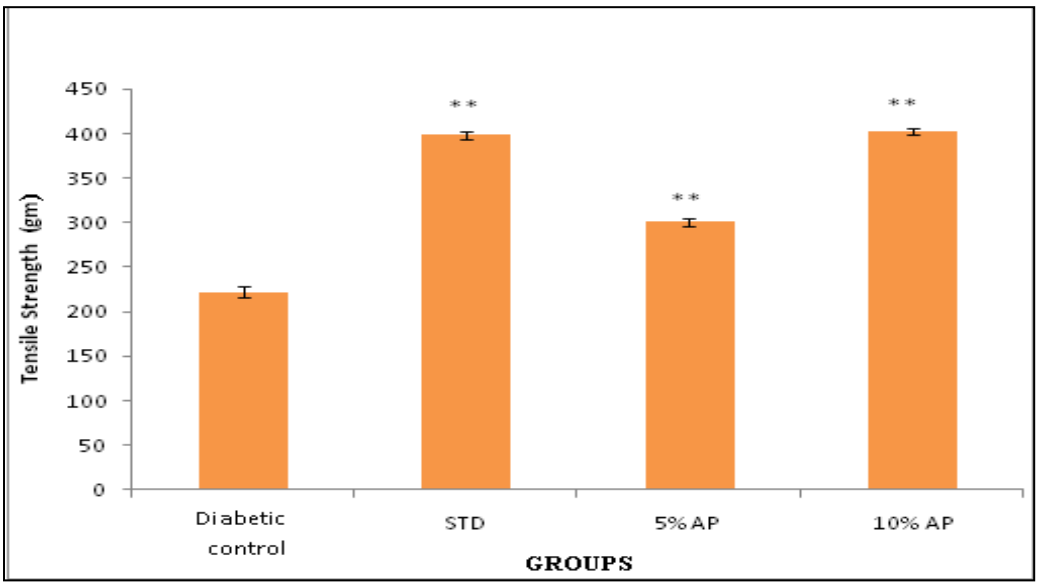


Figure 11: Tensile Strength in Incision Model in Diabetic Rats

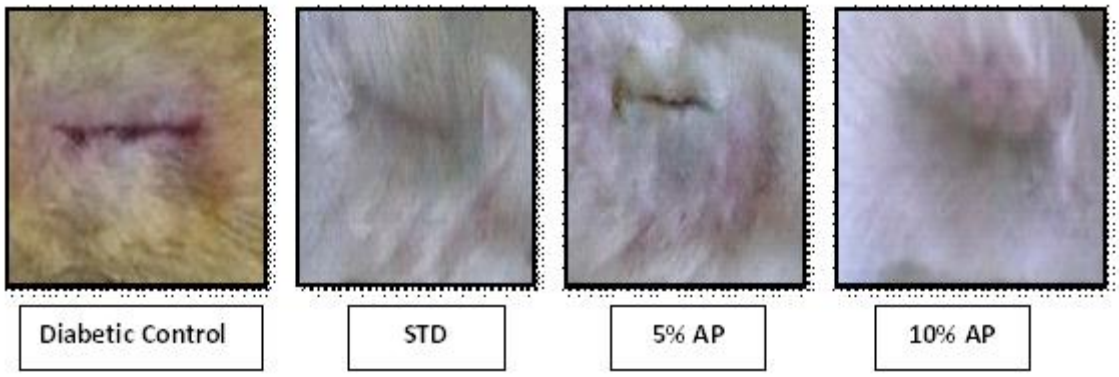
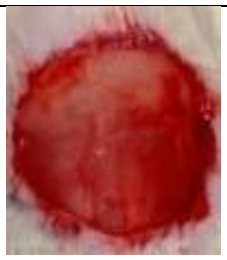
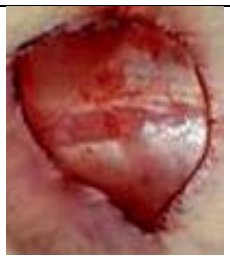
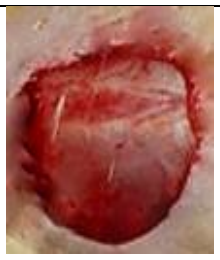
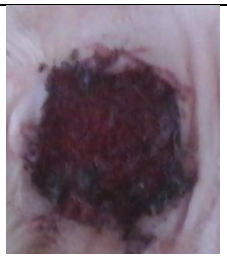
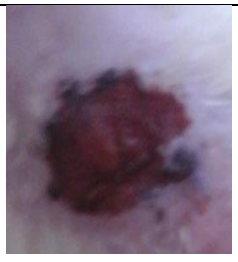
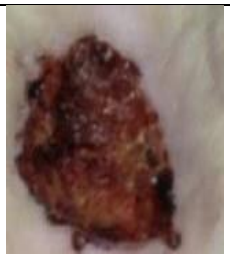
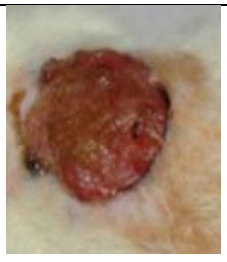


Figure 12: Tensile Strength in Incision Model of Diabetic Rats

DAYS	GROUPS			
	Diabetic Control	STD	5% AP	10% AP
0				
4				

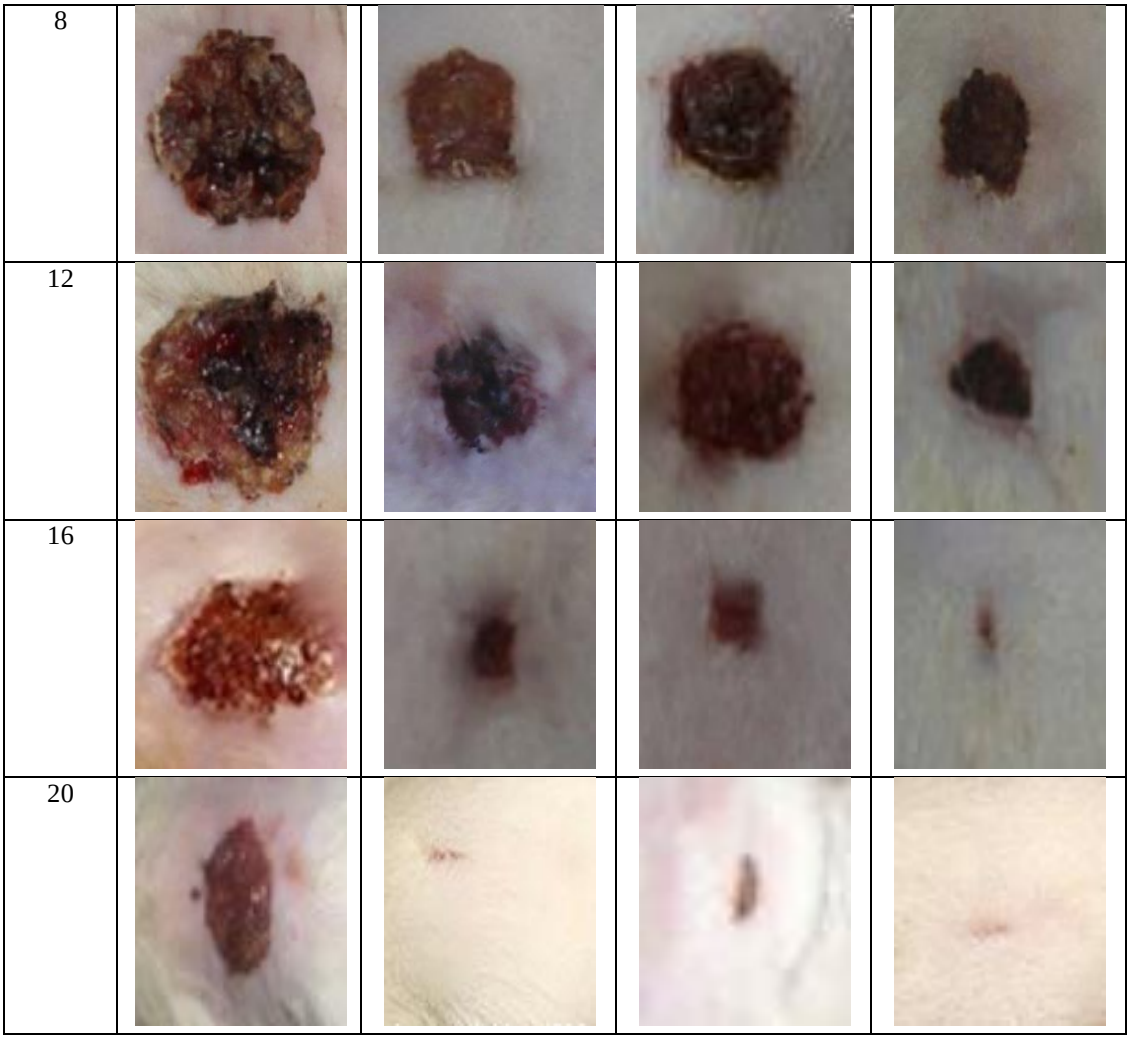


Figure 13: Process Wound Healing in Incision Model in Diabetic Rats

During Histopathological analysis of wound tissue we observed that there was less inflammatory changes, increase tissue proliferation as well as remodelling along with re-epithelization, increase collagen fiber along with low scar formation in diabetic animals treated with 5% and 10 % AP compared to diabetic control (Figure 14). The same findings were observed by wound healing property of topical application of ethanolic extract of *Michelia champca* flowers in diabetic rats[33].

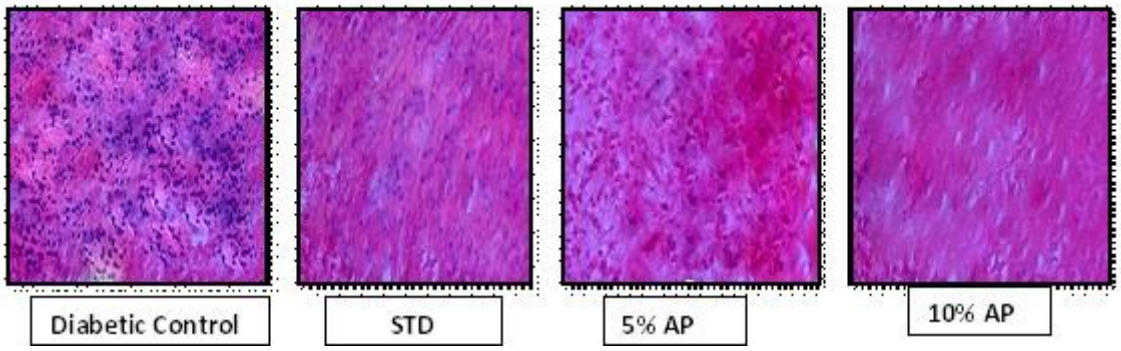


Figure 14 Histopathological Examination of Wound Membrane in Diabetic Rats

The phytochemical analysis of ethanolic extract of AP showed the presence of flavonoids, which has been documented to have free radical scavenging effect and antibacterial activity[34]. Moreover Flavonoids have shown to increase collagen synthesis, decrease degradation of collagen, acceleration of the conversion of soluble collagen to insoluble collagen, support the cross-linking, and inhibit catabolism. Flavonoids are also play major role in increase lymphatic drainage, decrease oxygen free radical and increase collagen synthesis to recover wound healing[35]. Therefore, wound healing potential of *Abrus precatorius* may be attributed due to presence of phytoconstituents present in the ethanolic extract of AP which may be either due to their individual or additive effect that speeds up the process most probably the proliferation phase of wound healing. Pal *et al.* (2009) proved the potential *Abrus precatorius* for antioxidant activity. Antioxidants reduce superoxide radical formation and prevent the damage of cells caused by free radical[36]. Thereforeplay major role in wound healing process. These results completely justify the folkloric use of *Abrus precatorius* for wound healing property. Also apart from just usefulness of *Abrus precatorius* leaves in simple wound healing process, it can be also useful in diabetic wound healing process. However further phytochemical studies are needed to isolate the active compounds responsible for the pharmacological activity. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of *Abrus precatorius* leaves.

CONCLUSION

The ethanolic extract of leaves of *Abrus precatorius* (AP) showed significant effect on wound healing activity in both normal and diabetic group of rats as compared with simple base and marketed preparation Nitrofurazone.

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