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Development and Evaluation of Surgical Sutures from *Agave americana* Linn. Var. *Americana*.

Sohani B. Solanke^{1*}, Santosh S. Bhujbal², Mukund G. Tawar³

1. Department of Pharmacognosy, Faculty of Pharmacy, Dr. Rajendra Gode Institute of Pharmacy, Amravati-444603

2. Department of Pharmacognosy, HOD, Padm. Dr. D.Y. Patil Institute of Pharmaceutical Science and Research, Pimpri, Pune-18

3. Principal, PR Pote Patil College of Pharmacy, Amravati-444603

ABSTRACT

Natural materials used to close wounds include flax, hair, grass, cotton, silk, pig bristles, and animal gut. Agaves were the source of many essential items due to its high tensile strength. Agave fibers were investigated for its potential use to close the topical incisions in rat. Sutures were developed i.e. Agave suture from the isolated fibers of *Agave americana* L. var. *americana* (Agavaceae) plant and were coated with the ethanolic extract of *Aloe vera*. These were evaluated for Physical properties which proved good tensile strength, handling properties, low tissue drag, acceptable knot security, low microbial infiltration, toxicity and faster incision wound healing in rats compared to commonly utilized surgical sutures from market. Wound healing was evaluated by observing pus formation, inflammation, incision wound closure period, tensile strength measurement and histo-pathological evaluation of healed wound tissue. All the mentioned tensile strength tests were performed by a tensile strength testing apparatus, especially designed and constructed for our purpose. According to findings Agave suture shown acceptable results and hence for can be considered for further research and utilization.

Keywords: Surgical suture, *Agave americana* L., *Aloe vera* L., incision wound healing.

*Corresponding Author Email sohanisolanke@rediffmail.com

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INTRODUCTION

Background Surgery has become an integral part of global health care, with an estimated 234 million operations performed yearly (Haynes A.B. et al., 2009). Surgical site infections are the third most commonly reported nosocomial infection and they account for approximately a quarter of all nosocomial infections. They have been responsible for the increasing cost, morbidity and mortality related to surgical operations and continues to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative preparation and antibiotic prophylaxis (Lilani S.P., et.al. 2005). Diabetes affects approximately 170 million people worldwide and by 2030 these numbers are projected to double (Brem H, et.al.,2004). Wound healing is a complex process that has attracted researchers over years to develop a surgical suture which should promote wound healing. Most of the literature evaluated suture material in relation to wound healing focuses on the suture's ability to strengthen the wound during the healing process, minimizing infection and achieving optimal cosmetic and functional results. Providing adequate mechanical support to maintain wound strength until the tissue is sufficiently healed to withstand stress and strain on its own critical to achieve both these aims. Ideally, the suture chosen to close the wound should make the wound site as strong as intact tissue. *Agave americana* consists of the lignocellulosic (Megiatto J.D., et.al.,2007) fibers and has the strength and other properties those inspired to develop the surgical suture. *Aloe vera*, a tropical cactus, has proved antimicrobial, anti-inflammatory (Vogler B.K.,1999) rapid wound healing activity (Megiatto J.D.,et.al.,2006) preventing adhesion (Kilic N., 2005) and efficiency in diabetic rats is reported by influencing phases such as inflammation, fibroplasia, collagen synthesis and maturation, and wound contraction. Topical application or oral administration of *Aloe vera* in wounds treatments shows similar effects (Chithra P., et. al, 1998; John P. Hegggers, et. al 1995). Therefore it has been selected for coating of suture.

The purpose of this study was to determine the influence of surgical sutures on wound healing in healthy and diabetic is checked (Missiry M.A., Gindy A.M., 2000). The sutures are developed from widely available *Agave americana* plants isolated (Reddy N., Yang Y., 2007). Fibers and were coated with a potent wound healing plant extract i.e. *Aloe vera* (Saha K.,et. al.,1997; Gal P., 2006; Davis R.H., 1989).

MATERIALS AND METHOD

Plant material:

The plants *Agave americana* L. var. *americana* (Family- Agavaceae) and *Aloe vera* (L.) Burm. f (Family- Liliaceae) was collected from Pimpri, Pune district in India in the month of January 2010.

The plants were identified and voucher specimens no. SOAAS01 and SOALOS03 respectively have been deposited in Botanical Survey of India, (BSI) Pune.

Extraction:

Agave americana leaves were washed with water and were boiled with 4% sodium hydroxide solution in water for 3 hours. Then the fibers were removed from solution and were washed with hot water thrice, to remove all extraneous matter from fibers. Then fibers were washed with the 2% EDTA solution to acquire strength. Fibers were removed, separated from each other and dried in shade. Yield of the fibers obtained was 8 % w/w. *A. vera* extract was prepared by maceration *A. vera* leaf gel (100gm) with ethanol and dried with vacuum evaporation. Yield of the *A. vera* ethanolic extract obtained was 1.5 % w/w.

Development of suture:

The tensile strength of the fibers was measured according to the USP 2004 using Tensile strength tester (make: Ubique and Capacity 200 kGF). Four fibers were braided to form a strong cord or yarn. The diameter was suitable to stitch the cut on skin was equal to the generally used suture for skin surgery i.e. silk no. 4-0. Dip Coating method was utilized for coating the yarns. Yarns were weighed & dipped in *A. vera* ethanolic extract up to 10% w/w. These were air dried and dipped in melted white bees wax to acquire a weight of $20\% \pm 5$ w/w. Diameter was suitable to stitch the cut on skin. Sutures were immersed in ethanol before surgery for sterilization. Sterility test for the sutures was performed according to the US Pharmacopoeia 2004 edition.

Evaluation of sutures:

The developed sutures (Test suture) were compared with a suture (Standard suture) generally used for skin surgery i.e. silk no. 4-0 (Merisilk, NW 5049, Ethicon division of Johnson & Johnson, Aurangabad).

General characteristics:

General characteristics included handling properties of suture. Diameter of the suture was checked using Digital vernier caliper (Radical Co Mitu-toyo). The tensile strength was checked using tensilometer (make: Ubique, Capacity 200 KGF).

Microbial Infiltration:

A single colony of *E. coli*, the bacterium used in this study, was removed from the master plate and then transplanted and sub cultured on standard methods agar at 37°C for 12 hours. The sub cultured bacterium was diluted (1×10^6 /ml) to produce a 250 ml solution. The 3 suture specimens were tied to 3 sterilized clips and were immersed in the solution for 10 minutes. After 10 minutes, a 1 cm portion at the end of each of the specimens was cut off to be used for the experiments. In **Experiment**

1, the sutures cut off from the 3 material specimens were placed onto standard methods agar plates and sub cultured at 37°C for 12 hours for observation. **In Experiment 2**, the sutures cut off from the 3 material specimens were diluted with a 1 ml tryptic soy broth. A 100µl of each of the diluted portions was then placed onto a standard agar plate and was sub cultured at 37 °C for 12 hours for later observation. For the quantification (counting colony), the spectrophotometry was used. Both experiments (standard methods agar and tryptic soy broth) involved 5 plates, respectively, for each of the 3 specimens.

Wound healing activity:

In vivo wound healing with sutures was checked using incision wound healing technique in normal healthy and diabetic rats.

Animals:

Male *Ratus norvegicus* rats (160-200 g) were obtained from National Toxicology Center, Pune, were housed as single animal per cage and maintained at an ambient temperature of 25±1⁰ C with humidity of 50±10% and a 12:12 dark: light cycle. All rats were allowed free access to food and water. The experiments were conducted according to the guidelines of the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Government of India, and approved by Institutional Animal Ethics Committee.

Wound healing activity in normal healthy rats:

Two groups (n=5) of rats as Control group using standard suture & test group using test sutures was made. Under general anesthesia paravertebral incisions of 2 cm were made through the abdominal skin and cutaneous muscles at a distance of about 1.5 cm from the midline on one side of the depilated back of the rat. After the incision, parted skin was kept together and stitched with respective sutures at 0.3-cm intervals and using a curved needle (No. 18). The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed and no local or systemic antimicrobials were used throughout the experiment. Observations were done daily until wound dries. When wounds were cured thoroughly the suture & fibers were removed on the day.

Parameters evaluated Singh (M., Pal, Sharma C.S., 2009) were- wound healing period, infection, inflammation, any other observations, tensile strength and histology of healed wound tissue. Pus is the aggregation of white blood cells with a liquid like consistency in the wound. Infection or pus formation was measured daily by gentle pressing the sides of wound to observe any extrusion out of the wound. '1' is designated for presence and '0' for absence of pus. The reddening was observed daily as inflammation and was traced on a transparent sheet with the permanent marker. The area

was measured as inflammation area. On the 7th day all rats were sacrificed and wounded skin tissue of 2 x 2 cm² dimensions was cut off and two of them were immediately fixed with 10% formalin for histological study and three tissue samples were utilized for tensile strength measurement.

Histological study:

The wound and surrounding tissues were fixed with 10% formalin, embedded in paraffin and sectioned. Sections were stained with hematoxylin and eosin (H&E). Three separate sections from each wound were examined by light microscopy using Binocular / Trinocular microscope (CXR II). Morphological findings including epithelialisation, cellular content (neutrophils, macrophages, and fibroblasts), collagen regeneration, and vascularisation were semi-quantitatively evaluated in coded slides and scored 0, 1, 2, 3 as in Table 1.

Table 1 : Explanation of used scale in the semi-quantitative evaluation of histological sections¹³

No.	0	1	2	3
Epithelization	Thickness of cut edges	Migration of epithelial cells	Bridging of the incision	Complete regeneration
PMNL Tissue	Minimum	Mild	Moderate	marked
macrophages	Absent	Mild	Moderate	Marked
Fibroblasts	Absent	Mild	Moderate	Marked
New collagen	Absent	Mild	Moderate	Marked
Neo-angeogenesis	Absent	Mild	Moderate	Marked
Centro-nucleated cells	Absent	Mild	Moderate	Marked

Determination of tensile strength:

The tensile strength test was performed using an apparatus specially designed and constructed for our purpose at our laboratory as shown in figure 1 on the same principle as thread testing in the textile industry.

Figure 1: Tensile strength tester , especially designed and constructed for our purpose



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Each tissue samples was placed on the middle of the board. The clamps were carefully attached to the skin tissue on the opposite sides, at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally on to the weightless pan with addition of same weight clay balls until the wound began to open. The amount of clay balls in the weightless pan was weighed and considered as an indirect measure of the tensile strength of the wound. Tensile strength was calculated using formula:- $\text{Tensile strength} = \text{Total breaking load} / \text{Cross-sectional area}$. The average of determination of tensile strength on the two paravertebral incisions on both sides of the animals were taken as the measures of the tensile strength of the wound for individual animal. The tensile strength increment indicates better wound healing.

Wound healing activity in diabetic rats:

Diabetes was induced by a single dose of 120 mg/kg Aloxan in normal saline by i.p. rout injection of a toxin specific for insulin-producing cells, in saline-sodium citrate buffer (Sigma, Inc., St. Louis, MO., pH 4.5). Blood glucose levels were checked using Blood glucose estimation kit (GR-102; TERUMO Co., Tokyo, Japan). Ten days after Aloxan injection, animals with blood glucose levels above 200 mg/dL were defined as diabetic and used in the study. All procedures were followed similar to the wound healing activity in normal healthy rats. The rats were sacrificed on 10th day after surgery for histological evaluation.

Statistical analysis:

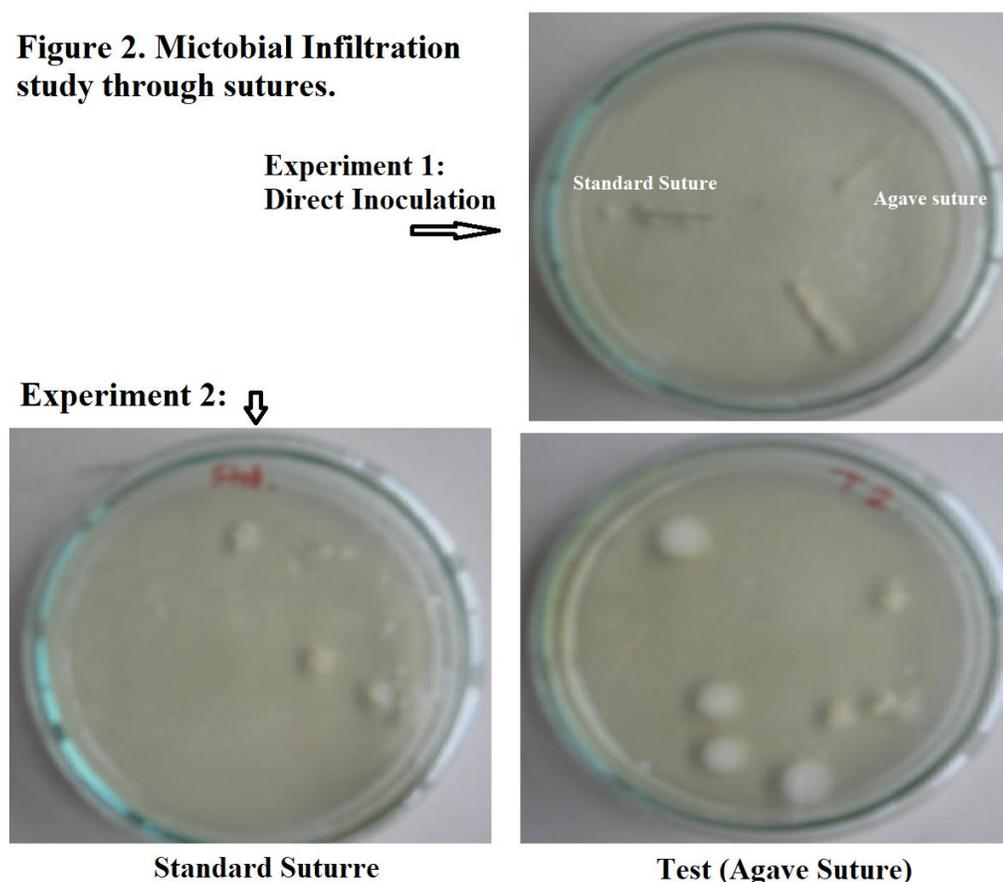
The data obtained are presented as mean \pm standard deviation (SD). All the results were compared by one-way ANOVA followed by unpaired t-test test. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

General characteristics:

(Figure 2) Test sutures were observed for general characteristics to have good handling properties, passes smoothly through tissue or has low tissue drag, performs knot security i.e. knots does not sleep out. Test (Agave) sutures had diameter of 1.8 ± 0.02 mm and tensile strength of 0.8 ± 0.04 Kg. *Aloe vera* coating was $10 \pm 0.5\%$ w/w base coat with $20 \pm 0.5\%$ w/w of final coat with diameter of suture 1.8 ± 0.02 mm. Statistically significant results observed in sutures without *Aloe vera* coat was $1.75 \pm 0.85^*$ and that of *Aloe vera* coated sutures as $7.0 \pm 1.41^*$.

Figure 2. Mictobial Infiltration study through sutures.



Experiment 1(Direct Inoculation): (Figure 2)

Little microbial infiltration was found in the sample of Agave suture and Standard (silk suture) suture in direct inoculation method. Standard suture shown 3.0 ± 0.04 microbial infiltration with 0.81SD and Agave Suture shown $1.75^* \pm 0.85$ SEM microbial infiltration with 1.70 SD. Where, n=4, Standard= silk suture, ns= $P > 0.05$ and * = $P < 0.05$.

Experiment 2:

Average numbers of colonies were calculated by counting on the 5 plates of each of the 2 sutures, i.e. Silk and Agave suture. Standard suture shown 26.4 ± 1.20 microbial infiltration with 2.70 SD

and Agave Suture shown $7.0 \pm 1.41^*$ microbial infiltration with 3.16 SD. Where, $n=4$, Standard=silk suture, $ns=P>0.05$ and $* =P<0.05$.

Incision Wound Healing:

Wound drying, closure period, healed tissue's tensile strength was observed by necked eyes. Wounds were healed earlier in test group than control group as shown in table 2 without any infection and inflammation. The wound closure was observed in 6.8 ± 0.2 days with 0.44 SD in case of control group, and 4.8 ± 0.37 and 0.83 SD in Agave test group. Where each value represents mean \pm SEM, ($n=5$). Normal skin tensile strength was 48.56 ± 5.37 lbs/cm with 9.31 SD. Test group (Agave) showed better tensile strength i.e. 31.06 ± 3.62 lbs/cm with 3.17 SD than Control group i.e. 18.76 ± 1.83 lbs/cm with 6.27 SD. Each value represents mean \pm SEM, ($n=3$), $ns=P>0.05$, $* =P<0.05$. It proves better strength to repair surgical tissues.

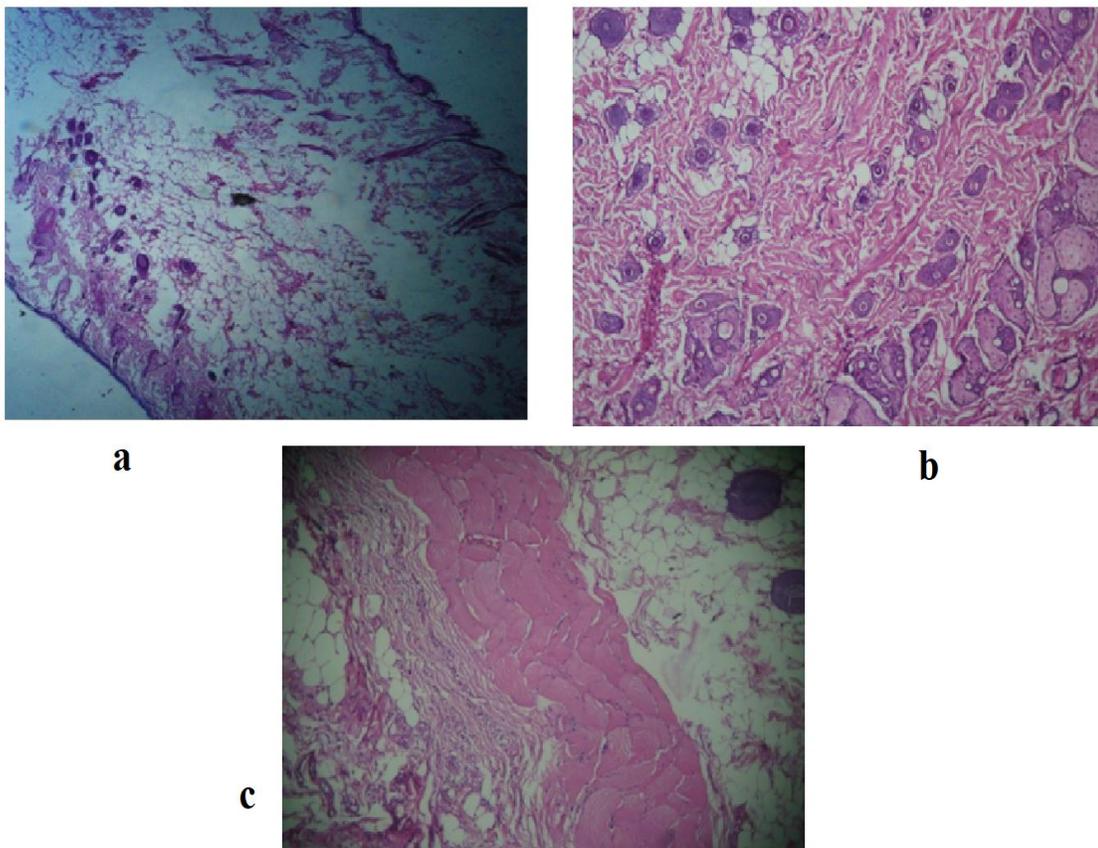
Table 2: Histopathology of Healed wound tissue of normal rats group (i.e. on 7th day).

Sr.No.	Characteristics	Control group	Test group	Normal skin
1	Epithelization	3	3	0
2	PMNL	2	1	0
3	Tissue macrophages	3	3	1
4	Fibroblasts	3	3	0
5	New collagen	3	3	0
6	Neo-angiogenesis	1	3	0
7	Centro-nucleated cells.	1	1	1
8	Scar	3	1	0

Where, $n=3$.

These results were partially assured with histological evaluation as observed in the figures 3. Presence of minimum macrophages indicates less to no infection in the tissues. The collagen content was more in test group than control group, seen as a dark band around wounded site. Scar is observed as damaged tissues were minute in test group.

Figure 3: Histomorphology of Rat abdominal skin on seventh day.
a: rat normal skin, b: control group rat skin, c: *Agave* test group rat skin



Wound healing activity in diabetic rats:

Diabetes was ensured in rats on tenth day after Aloxan administration. The sugar level was above 200 mg/dL in all the rats. Then the experimental procedures followed. Wound healing was delayed in these rats compared to non-diabetic rats, due to diabetes. The wound closure was observed in 9.60 ± 0.24 days with 0.54 SD in case of control group, and 7.80 ± 0.37 and 0.83 SD in *Agave* test group. Where (n=5).

But wounds were healed earlier in test group than control group with comparatively less infection and inflammation. Normal diabetic rat's skin tensile strength was 37.75 ± 2.52 lbs/cm with 4.36 SD. Test group (*Agave*) showed better tensile strength i.e. $23.72 \pm 2.59^*$ lbs/cm with 4.48 SD than Control group i.e. 18.20 ± 1.75 lbs/cm with 3.03 SD. Each value represents mean \pm SEM, (n=3), ns=P>0.05, * =P<0.05. It proves better strength to repair surgical tissues in diabetes. These results are partially assured with histological evaluation (table 3) as observed in the figures 4. The wound was not even well closed in control group as shown in figure 4.

**Figure 4: Histopathology of Healed wound tissue (i.e. on 10 th day) in diabetic rats.
a: rat normal skin, b: control group rat skin, c: *Agave* test group rat skin**

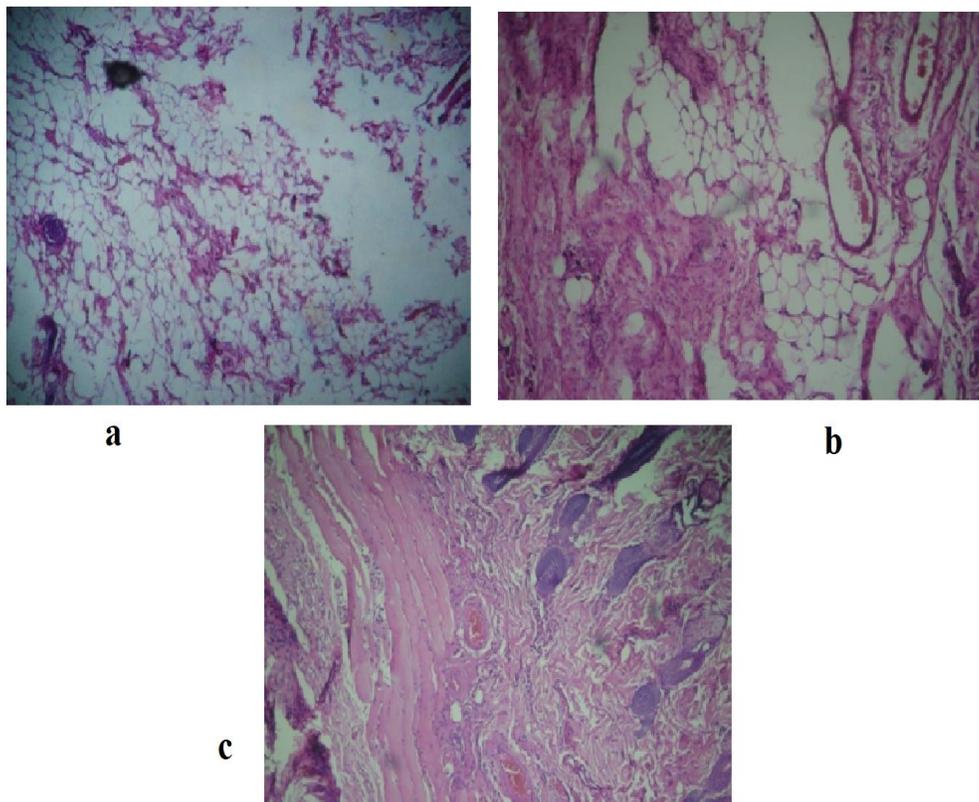


Table 3: Histopathology of Healed wound tissue (i.e. on 10th day) in Diabetic rats.

Sr.No.	Characteristics	Diabetic Control group	Diabetic Test group	Normal skin (without wound)
1	Epithelization	3	3	0
2	PMNL	2	1	0
3	Tissue macrophages	3	3	1
4	Fibroblasts	3	3	1
5	New collagen	3	3	0
6	Neo-angiogenesis	1	3	0
7	Centro-nucliated cells.	1	1	1
8	Scar	3	1	0

Where, n=3.

CONCLUSION

The wound healed faster in case of *Agave* suture group by achieving better tissue tensile strength. The effects may be due to the reported hypoglycemic effects of the aloe gel. Therefore the yarns made up of the above plants were coated with the *A. vera* gel ethanolic extract (Vogler B.K.,1999; Subramanian S.K.S., Arulselvan P., 2006). All the results indicate a better wound healing with the *A. americana* suture by achieving maximum tissue tensile strength in relatively less period than Standard sutures. *Agave* sutures dried faster. Hence, this novel approach to use natural fibers as

suture can be utilized for the rapid and better quality wound healing along with inexpensive manufacturing cost.

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