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Original Research Article (Experimental)

In-vivo study of tissue reaction to *Crotalaria pallida* and *Sansevieria roxburghiana* fibers

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ABSTRACT

Background: A suture material producing least tissue reaction is considered as ideal. Other characteristics like tensile strength, capacity to sustain sterilization process enhance its acceptability. In the present situation there is a need to reascertain the relevance and utility of these materials. Among the suture materials mentioned by Sushrutacharya, *Moorva* (*Sansevieria roxburghiana*) and *Shana* (*Crotalaria pallida*) have been showed insignificant tissue reaction in operated cases of inguinal hernia. An experimental study to confirm the extent of tissue reaction in deeper planes is needed before extending the use of materials in the deep tissues.

Objective: The objective of the study was to analyze deep tissue reaction and tensile strengths of plant fibres extracted from *Crotalaria pallida* and *Sansevieria roxburghiana*.

Materials and methods: The study was conducted on 18 albino rats, 3 groups of 6 rats each for a period of 21 days inserting the suture materials in deeper tissue, studying histopathology changes of the deeper connective tissues, hydroxyproline content and blood parameters on 7th, 14th, 21st days of the study. The tensile strength of the two materials was also assessed on 7th day in three different conditions. Statistical analysis was carried out using paired and unpaired *t* tests.

Results: *S. roxburghiana* had least tissue reaction. *C. pallida* showed greater tensile strength in comparison to *Moorva*.

Conclusion: *C. pallida* can be used for deep tissue approximation because of its moderate tissue reaction and tensile strength, successive increase in hydroxyproline content and its capacity to sustain sterilization.

1. Introduction

The goal of suturing a surgical wound is to close the dead space until healing re-establishes the tensile strength of wounds, minimize the risk of bleeding and infection, approximate skin edges for better cosmetic and functional values. At the same time suture material, would act as a foreign body and induce reactionary changes. An ideal suture material should achieve the goals with least reactions. Sutures are classified as natural vs. synthetic, absorbable vs. non-absorbable, and multifilament vs. monofilament, braided vs. twisted [1].

Acharya Sushruta has described different suturing materials of plant and animal origin. *Shana* (*Crotalaria pallida*) and *Moorva* (*Sansevieria roxburghiana*) are important plant substances among them as the natural fibers have more tissue acceptance and biotolerance than synthetic fibers and moreover, with the growing demand for healthier and environment- friendly products, *Shana* and *Moorva* were selected for this study. Acharya Sushruta, has described the comparatively less intense reaction of a plant substance in the body [2].

An earlier report had shown that the plant *Shana* (*C. pallida*) has antimicrobial and anti-inflammatory effects [3,4] and phytochemical analysis showed the following chemical compounds like

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alkaloids, flavonoids, terpenoids, saponins, phenols, steroids, tannins. *Moorva* (*S. roxburghiana*) with its antimicrobial and analgesic effects [5,6] after phytochemical studies revealed the presence of carbohydrates, saponin, flavonoids, phenols, alkaloid, anthocyanin and -cyanin, glycosides, proteins and phytosterols.

Among the suture materials listed by Sushrutha, horse hair, hasn't gained popularity may be due to its low tensile strength and its biodiversity [7]. *Moorva* and *Shana* fibers have been studied previously for skin suturing in patients operated for inguinal hernia in our institution [8,9] which has shown insignificant tissue reaction. Hence, this study has been undertaken with an objective to study the reaction of the deeper tissue to the two suture materials by analyzing the histopathology, hematology and biochemical changes, estimating hydroxyproline content and their tensile strengths.

2. Materials and methods

2.1. Materials

2.1.1. Collection of plants and preparation of trial suture materials

Ideal time for collection of Shana (*C. pallida*) [10] is September to February. Mature *C. pallida* plants were collected from an edge of field, which are erect, about 1 cm thick and with minimum branches. Plants were cut three inches above ground level, discarding its branches, leaves and terminal parts. Fibers were extracted according to present methods used for plant fiber extraction. Mature stem was collected, washed thoroughly in running water. After retting for about 8–10 days, fibers were procured and stored in pollution-free environment. Single strands of fibers were separated with gentle sliding motions from one end to other end of stem.

A fully grown *Moorva (Sansevieria roxburghiana)* [11] leaf was collected. Leaf was subjected to pressure heating for 35 min, given three–four longitudinal slits and soaked in clean water for 7 days. The leaf got softened and bundle of fibers could be easily separated from parenchyma. These bundles were further thoroughly rinsed in running water to get silky, thin fibers.

Single strands of *Shana* and *Moorva* obtained were rinsed in running water, placed on dry clean cloth, dried in shade for 3–5 h. Stored in polythene cover and preserved. Five strands of *Shana* and *Moorva* were entwined together to prepare multifilament *Shana* and *Moorva* suture materials which were placed on dry clean cloth, dried in shade for 3–5 h. They were stored in polythene cover and preserved. Before insertion into albino rats the monofilaments and multifilaments were autoclaved.

2.1.2. Experimental animals

Wister albino rats of weight 200 ± 50 g body weight were procured from animal house attached to Pharmacology laboratory at the institutional Research Centre. Animals were maintained at standard laboratory conditions such as temperature at 25 ± 2 °C, humidity of 55–60% and natural day and night cycle. They were fed with Amrut brand rat pellet feed supplied by Sri Durgha Feeds, Bangalore and tap water was given *ad libitum*. The study protocol was approved by Institutional Ethics Committee and principles of laboratory animal care guidelines were followed throughout the experimentation.

2.2. Methodology

Albino rats taken randomly from animal house for the study were grouped into three groups. They were placed in three separate cages consisting of six rats each painted with picric acid on head, neck, body, tail, forelimb and the last one was left without any mark for the purpose of their identification respectively. Trial group A rats: autoclaved *Shana* fibers. Trial group B rats: autoclaved *Moorva* fibers. Standard group C rats: autoclaved Cotton fibers.

2.3. Surgery

2.3.1. Pre-operative procedures

Necessary sterile instruments were kept ready. The rats were shaved from nape of neck to the midback region. The prepared part was cleaned with spirit. During surgical procedures the prepared rat was placed on a platform cleaned with Dettol.

2.3.2. Surgical procedure

Each rat was anesthetized with ketamine in a dose of 50 mg/kg body weight and xylazine in a dose of 3 mg/kg body weight. Four incisions in total were made on upper left, upper right, lower right and lower left regions of prepared part (Fig. 1A). A sterilized monofilament fiber of *Shana* of about 1 cm length was placed in right half in each of first three incisions. A sterilized multifilament fiber of five strands of *Shana* of about 1 cm length was placed in left half in each of first three incisions. A sterilized strand of *Shana* of about 4 cm was inserted into SITE4 (lower left region). The wounds were closed with cotton suture, cleaned with Dettol. The operated rat was placed in a separate cage under observation for 10 min, after which it was placed in its original cage. The similar procedure was followed for rats of groups *Moorva* and Cotton.

2.3.3. Observations on re-incisions

Blood for hematology and serum for biochemical analysis were drawn from retro-orbital puncture of rats of all three groups. Under previously mentioned aseptic precautions, after anesthetizing the rat with ketamine and xylazine, re-incision was given at SITE1 (upper left incision), SITE2 (upper right incision) and SITE3 (lower right incision) respectively on 7th, 14th and 21st days of the study. The fibers along with the tissue in contact was excised, cleaned with normal saline, weighed for histopathology studies which was stored in a mixture of 10% formalin in different sterile containers, processed further and stained by using haematoxylin and eosin stains. A part of excised tissue was homogenized and concentration of hydroxyproline was estimated. The closure of wounds in SITE1, SITE2, SITE3 was made with sterilized cotton thread. Wound care was given.

The SITE4 i.e. lower left incision was re-incised on 7th day and inserted strand of 4 cm was removed, washed with NS and placed for measurement of tensile strength. SITE4 was sutured with cotton thread and wound care was given. Rats of all three groups were sacrificed after 21 days of study. The histopathology study of tissue reaction, hydroxyproline estimation was analyzed to draw conclusions.

2.3.4. Tensile strength measurement

An apparatus was devised which comprised of two vertical rods, fixed to two horizontal rods arranged in a shape of alphabet H, at their upper and lower levels. A small pulley was attached to the upper horizontal rod at the center, to which a cotton thread was tied, the free end of that thread was tied with a surgical knot to the suture material in test.

An empty NS bottle was attached to IV drip set for controlled flow of liquid into the bottle. That bottle was tied to another cotton thread, to which other end of the suture to be tested would be tied with a surgical knot (Fig. 1B).

Once this apparatus was set, the liquid would flow under control into the bottle. At a point where the weight of bottle would exceed such that the suture material in test would break, was considered as tensile strength of that particular suture material. Thus tensile

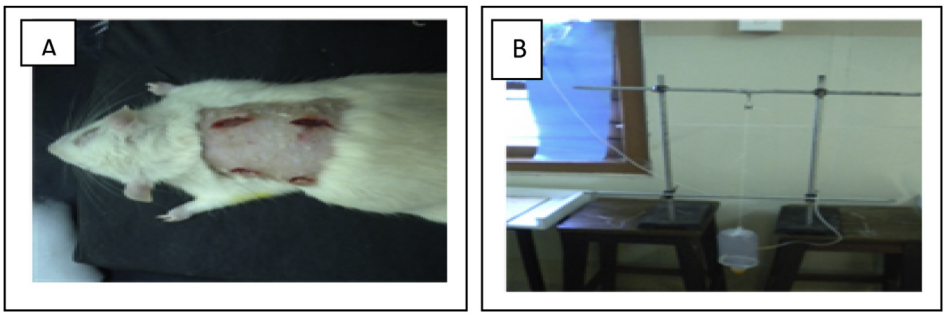

Fig. 1. A) Incisions on the back of the albino rat. B) Tensile strength measuring apparatus

strength was measured for single strands of *Shana*, *Moorva* and cotton in three different conditions i.e. non-autoclaved, autoclaved, inserted strands.

3. Results

3.1. Histopathology

3.1.1. On 7th day

Shana: Mild to moderate vascular response, mild active and chronic inflammatory cell infiltration, collagen fibers present, medium granulation tissue- indication of mild to moderate inflammatory reaction (Fig. 2A and B) was noted.

Moorva: Mild vascular response, mild active and chronic inflammatory cell infiltration, presence of giant cells indicates end stage of inflammation collagen fibers present, medium granulation tissue – an indication of mild to moderate inflammatory reaction (Fig. 2C and D) was observed.

Cotton: Mild to moderate vascular response, moderate active and chronic inflammatory cell infiltration, presence of giant cells indicates end stage of inflammation collagen fibers present, medium granulation tissue-indication of mild to moderate inflammatory reaction was noted in control.

3.1.2. On 21st day

Following changes were noted on 21st day in each groups:

Shana: Moderate vascular response, mild active and chronic inflammatory cell infiltration, presence of granuloma indicating end stage of inflammation by encapsulation, medium granulation tissue-indication of mild to moderate inflammatory reaction (Fig. 2E and F).

Moorva: Mild vascular response, moderate active and chronic inflammatory cell infiltration indicates a second episode of inflammation, presence of small granuloma, thick granulation tissue-indication of moderate inflammatory reaction (Fig. 2G and H).

Cotton: Moderate to high vascular response, mild active and chronic inflammatory cell infiltration, presence of large granuloma indicating end stage of inflammation by encapsulation, thick dense granulation tissue- indication of severe inflammatory reaction.

3.2. Hematology

3.2.1. White Blood Cells

There was a successive decrease in mean total leucocyte count in 1st, 2nd, 3rd weeks of the study respectively for both *Shana* and *Moorva* groups. The presence of *Shana* and *Moorva* in deep tissues produce mild inflammatory reaction in the initial days of placing the suture, doesn't produce significant changes in WBCs.

In the 1st week, there was an increase in mean total leucocyte count of both *Shana* and *Moorva* groups in comparison to cotton suggesting more tissue reaction of both groups. Unpaired *t* test is statistically significant in the 1st week of *Shana* and *Moorva* respectively in comparison to that of cotton fibers.

In the 3rd week, increase in mean total leucocyte count of *Moorva* group in comparison to cotton is suggestive of moderate inflammatory reaction in comparison to cotton. Unpaired *t* test is statistically significant for *Moorva* in comparison to cotton. Decrease in mean total count of WBCs was noticed in *Shana* group in comparison to cotton suggestive of mild tissue response of *Shana* group to that of cotton (Table 1).

3.3. Biochemistry

3.3.1. Total proteins

A successive increase of mean total proteins in 1st, 2nd, 3rd weeks respectively for all 3 groups was observed. Paired *t* test is statistically significant for all three groups.

In the 1st week, the mean total proteins of both *Shana* and *Moorva* groups were lesser in comparison to cotton. Unpaired *t* test is statistically insignificant for *Shana* and *Moorva*.

In 3rd week, mean total proteins of both trial groups were increased in comparison to cotton. Unpaired *t* test statistically significant in third week for all three groups (Table 1).

3.3.2. C-Reactive Protein

A successive decrease in mean CRP value was seen in 1st, 2nd, 3rd weeks respectively for *Moorva* group. Paired *t* test was statistically significant for *Shana* and cotton.

In 1st & 3rd weeks of the study mean CRP value of *Shana* and *Moorva* group was lesser than cotton, Unpaired *t* test was statistically insignificant for all three groups in 1st and 3rd weeks (Table 1).

3.4. Tissue hydroxyproline

There was successive increase in mean hydroxyproline level in 1st, 2nd, 3rd weeks respectively in *Shana* and cotton group. Successive decrease in 1st, 2nd, 3rd weeks respectively in *Moorva* group was observed. Paired *t* test was statistically significant for *Moorva* and cotton.

In 1st week the hydroxyproline content was lesser in Shana group in comparison to cotton, in *Moorva* group it was more in comparison to cotton. Unpaired *t* test was statistically significant in *Shana* and *Moorva* groups and also in *Moorva group* in comparison to cotton.

In 3rd week mean hydroxyproline content of *Shana* and *Moorva* group was more in comparison to that of cotton. Unpaired *t* test was statistically insignificant for all three groups in 3rd week (Table 2).

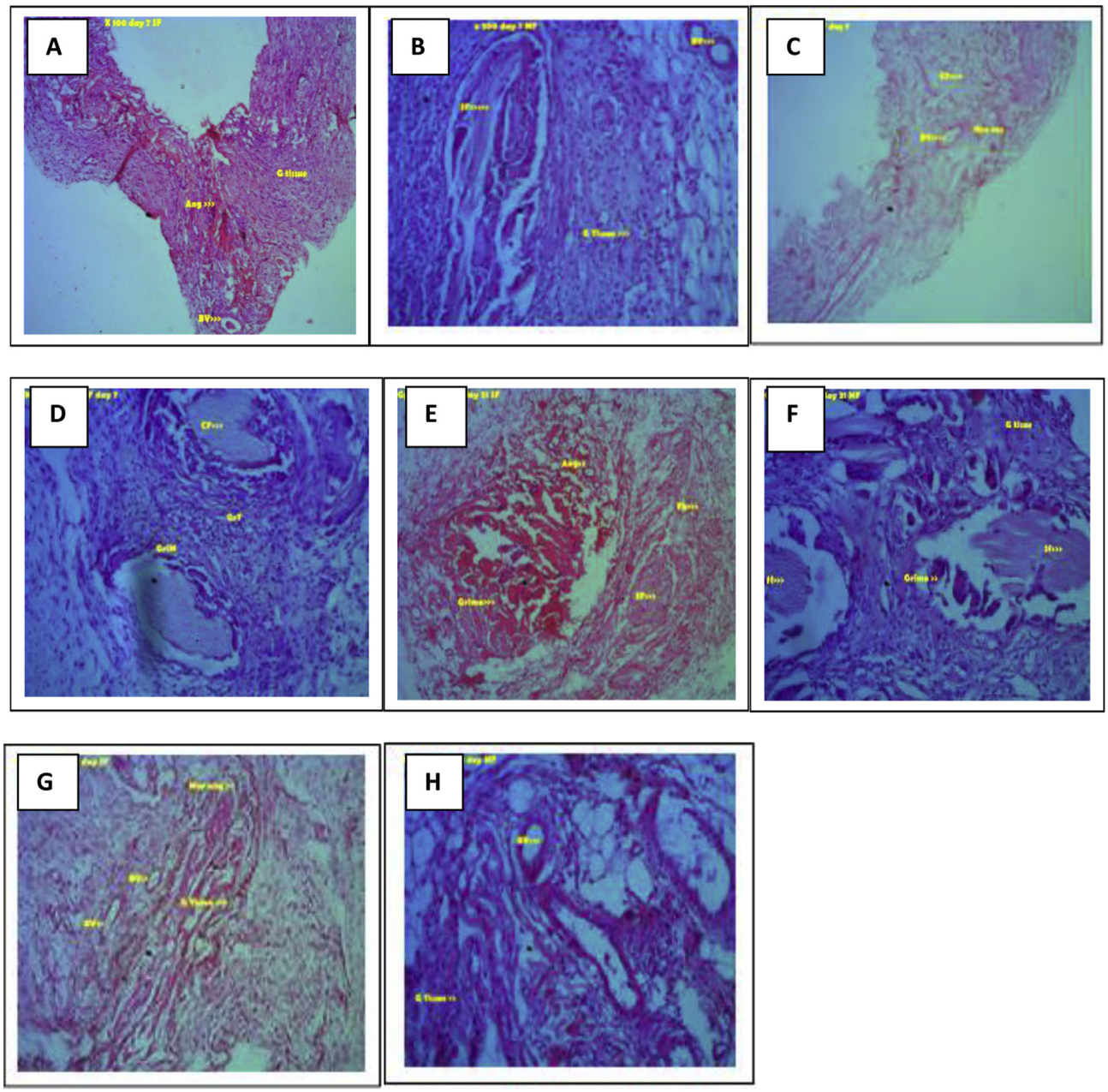

Fig. 2. Histopathology slides, H & E stains, A) and B) On 7th day *Shana* single and multiple fibers with granulation tissue, angiogenesis, blood vessels suggesting mild-moderate inflammation; C) and D) On 7th day *Moorva* single and multiple fibers with neovascularization, collagen fibers, blood vessels, granulation tissue suggesting mild inflammation, granuloma suggesting end stage of inflammation by encapsulation. E) and F) On 21st day *Shana* single and multiple fibers with angiogenesis, mild granulation tissue, fibroblast suggesting mild-moderate inflammation, granuloma suggesting end stage of inflammation; G) and H) On 21st day *Moorva* single and multiple fibers with moderate granulation tissue, neovascularization, blood vessels suggesting moderate inflammation.

3.5. Tensile strength

Mean value of tensile strength was found to be raised in autoclaved fibers of *Shana* and cotton single fibers group, than the non-autoclaved. Mean value of the tensile strength was found to decrease when inserted fiber was removed after one week for all three groups. Mean value of *Moorva* single fiber group was found to be decreased in autoclaved than that of non-autoclaved fibers (Table 3).

3.6. Observations

On 7th day: Wound health of both trial groups were good with no discharge, no edema in surrounding area. On re-incision of wounds, *Shana* material thickness decreased with no changes in length, about 1–2 cm thick tissue was adhered to the material. *Moorva* material was hardly visible with both thickness and length being decreased, about 0.5–1 cm thick tissue was adhered to the material.

In cotton group there was discharge from wounds and edema in surrounding area in two rats. On re-incision of wounds the suture material was clearly visible with about 2–3 cm tissue adhered to it, no changes in length and thickness of the material were observed.

On 21st day: Wound health of both trial groups were good with no discharge, no edema in surrounding area, good hair growth. On re-incision of wounds *Shana* material had an encapsulation which

Table 1
Mean hematological and biochemical parameters of study groups at different time intervals

Parameter	Groups	7th day	14th day	21st day
Total count WBC	A	13.22 ± 3.31#	12.91 ± 1.05	12.93 ± 1.37#
	B	17.46 ± 1.23##	17.50 ± 1.77	15.81 ± 1.22#
	C	5.38 ± 0.55	13.16 ± 1.17	12.04 ± 0.70
Total protein	A	8.43 ± 0.08	8.56 ± 0.12	8.71 ± 0.04# *
	B	8.28 ± 0.12	8.66 ± 0.08	8.85 ± 0.04# @ **
	C	8.53 ± 0.10	8.7 ± 0.17	8.7 ± 0.17*
C-Reactive Protein	A	1.04 ± 0.05	1.05 ± 0.03	1.09 ± 0.05
	B	0.76 ± 0.008	0.92 ± 0.10	0.86 ± 0.06*
	C	1.13 ± 0.24	0.91 ± 0.07	0.90 ± 0.15

Mean ± SE (n = 6); Unpaired *t* test for comparing standard with trial groups at different time periods; Paired *t* test for comparing 7th day with different time periods. # Significant difference with standard group, @ Significant difference with trial group, * Significant difference with 7th day, $^{*,\#,@}$ $p < 0.05$, $^{**,\#\#,@@}$ $p < 0.01$. A = *Shana*, B = *Moorva*, C = Cotton. Due to error during lab investigations, Group B WBC 7 days mean of 5 rats, Group C WBC 14, 21 days mean of 5 rats were computed.

Table 2
Mean hydroxyproline of study groups at different time intervals

Parameter	Groups	7th day	14th day	21st day
Hydroxyproline	A	8.16 ± 2.51	9.39 ± 2.28	24.13 ± 5.19
	B	53.4 ± 11.85# @@ **	22.74 ± 8.15	22.72 ± 3.92
	C	21.89 ± 6.71**	16.32 ± 5.69	15.99 ± 5.24

Mean ± SE (n = 12); students *t* test for comparing standard with trial groups at different time periods; Paired *t* test for comparing 7th day with different time periods. # Significant difference with standard group, * Significant difference with 7th day, $^{*,\#,@}$ $p < 0.05$, $^{**,\#\#,@@}$ $p < 0.01$. A = *Shana*, B = *Moorva*, C = Cotton. Due to error in storage of sample, 7 days mean of group A 11 rats and group B 9 rats, 21 days mean of group C 8 rats were computed.

Table 3
Mean tensile strength of study groups in different conditions

Groups	Non autoclaved	Autoclaved	Inserted and removed
A	0.1038 ± 0.0127*	0.1186 ± 0.0152@	0.0788 ± 0.009
B	0.0685 ± 0.0002*	0.0512 ± 0.001@	0.0468 ± 0.002**
C	0.9964 ± 0.0186*	1.1209 ± 0.134	0.1575 ± 0.0053

Mean ± SE (n = 6); paired *t* test for comparing study groups in different conditions; * Significant difference with non autoclaved group, @ Significant difference autoclaved groups, $^{*,@}$ $p < 0.05$, $^{**,@\ @}$ $p < 0.01$ A = *Shana*, B = *Moorva*, C = Cotton.

was moderate in comparison, about 0.5 cm thick tissue adhered with no changes in length and thickness of material. *Moorva* encapsulation was hardly present, less than 0.5 cm thick tissue adhered, length and thickness of the material had decreased drastically.

In cotton group there was discharge from wound, edema in surrounding area, wound dehiscence in one rat, hair growth present only in five. On re-incision of wound, encapsulation was markedly present, about 1 cm thick tissue was adhered to it, no changes in length and thickness of the material were observed.

4. Discussion

Sushrutacharya utilised naturally available materials for wound closure inspite of lack of manufacturing and synthetic development of suture materials in those days. Today, there is a need to promote these suture materials with a more research based scientific approach. In this study, two materials *Shana* and *Moorva* have been studied with an intention of having a cost effective natural suture material. The observations during the 21st day of the study in group A revealed an encapsulated mass with *Shana* material at its centre, may be hinting the non-absorbable property of the material which requires further studies to be concluded.

White blood cells serve three functions in inflammation phagocytosis, secretion of chemicals to cause local signals to induce or stop inflammation, immunity to help fight infection. In an inflammatory phase, acute inflammatory foreign body reaction peaks between 2–7 days. Initially polymorphonuclear cells, leucocytes, monocytes presence is predominant. By fourth day, mononuclear cells predominate and fibroblasts appear. By seventh day mature fibroblasts are present. By tenth day foreign material becomes encapsulated in a fibrous mantle. If implanted material is non-absorbable, at this point no further tissue reaction is observed [12]. Absorbable materials elicit a second boost of inflammatory reaction at the time of their resorption [13].

No significant raise in total count of leukocytes was seen in both trial groups, suggesting mild tissue reaction which is an ideal feature of a suture material. In 1st week *Shana* and *Moorva* produced a tissue reaction when inserted into deep tissues. Hence, both trial materials are safe to be used as external skin suture material since they will be removed by seventh day. 3rd week raise in total count results suggest that *Moorva* has more inflammation inducing and retaining capacity than cotton. So, further studies on absorbable property of *Moorva* are required. *Shana* didn't show any further reaction. Hence, *Shana* can be used in deep tissue requiring a long-term approximation.

Increase in total protein levels, especially Haptoglobin, a subvariety of serum globulin suggests response to stress, acute inflammation, tissue necrosis [14]. Increase in albumin levels suggest haemoconcentration.

Successive increase in total protein levels of all 3 groups suggests inflammation, hence both trial materials are unsafe for use for suture in deep tissues. In 1st week there was increase of total protein in cotton group than the trial groups, hence *Shana* and *Moorva* are safe for use for suture in deep tissues in comparison to cotton. In 3rd week both trial groups had raised levels of total proteins, suggesting a second boost of acute inflammation. Hence *Shana* and *Moorva* cannot be used for the deep tissue suturing requiring a long-term approximation.

C-Reactive Protein is elevated in response to trauma, inflammation, infections and decreases with resolution of condition [15]. CRP is used to monitor various inflammatory states. CRP binds to damaged tissue, nuclear antigens, certain pathogenic organisms in a calcium-dependent manner.

There was increase in CRP levels of *Moorva* and cotton groups, possibly suggesting a response to tissue trauma. In 1st and 3rd weeks there was no raise of CRP levels in both trial groups in comparison to cotton, hence *Shana* and *Moorva* are safe for use as sutures in deep tissues.

Hydroxyproline has an essential role in collagen stability without which no functional collagen fibers appear in extravascular spaces. It is also found in small amounts in elastin, acetylcholinesterase, the c1q component of complementary system, macrophage scavenger proteins, ectodysplasin A.

There was a successive increase in hydroxyproline levels of both trial groups, hence *Shana* and *Moorva* are safe for suturing deeper

tissue planes, as hydroxyproline is the important component of tissue collagen. In 1st and 3rd weeks of the study, hydroxyproline levels were raised in both trial groups than cotton group. Hence, *Shana* and *Moorva* are safe for deep tissue suturing as their insertion elevated the hydroxyproline content in the tissues which is very essential for fibrosis of the wound.

Tensile strength is the measured force in pounds, that the suture will withstand before it breaks. After autoclaving, decrease in tensile strength of *Moorva* single filament was observed, whereas increase in tensile strength was found in *Shana* and cotton single filaments. Mean value of the tensile strength was found to decrease when inserted filament was removed after one week for all three groups. Hence *Shana* is capable to withstand autoclaving and is having moderate tensile strength.

Standardization of preparation of *Shana* and *Moorva* suture materials with the aid of advanced technology. A long term detailed study on tissue absorbability and tensile strength of *Shana* and *Moorva* suture materials. An *in-vitro* hem compatibility test to determine the biocompatibility of the sutures.

5. Conclusion

The observations and the results obtained in this study indicate that in terms of least tissue reaction *Moorva* (*S. roxburghiana*) shows better efficiency than *Shana* (*C. pallida*). In terms of good tensile strength *Shana* was more efficient than *Moorva*.

Bearing the ideal suture characters in mind, it can be concluded that *Moorva* can be used as a deep tissue approximation material due to its least tissue reactivity, good biocompatibility. *Shana* can be used for deep tissues requiring long term support due to its moderate tensile strength, successive increase in hydroxyproline content and capacity to sustain sterilization.

Conflict of interest

None declared.

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