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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