EFFECT OF FEEDING WITH HERB OF ERYTHRINA VARIEGATA TO BIOCOMPATIBILITY OF THE COCOON FIBER OF WILD SILK MOTH ATTACUS ATLAS FOR FUTURE APPLICATION AS BIOCOMPATIBLE OF SILK SUTURES

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ABSTRACT

Objectives: Silk is biocompatible as biomaterial and has been used commercially as sutures. More interesting properties of the silk are that the mechanical properties exceed all natural polymer and synthetic materials. In this research, a type of silk suture is being developed from species of Attacus atlas to obtain better biocompatible sutures. A. atlas is a species of silk moth that consume not only single types of leaves. The Quality of cocoon fiber then can be arranged base on the types of the leaves that is consumed. Better biocompatibility sutures comparing with recent commercial silk sutures which is not biocompatible, can be achieved by feeding with variety types of leaves. Silk suture that already established in the market is a base product of Bombyx mori species of silk which is only consume one type of leaf (mulberry leaves).

Methods: In this research, the A. atlas cocoon was produced by feeding with herb Erythrina variegata. The microstructure was observed, element composition as well as biocompatible sutures was investigated.

Results: A high composition of kalium (K) as well as chloride (Cl) is identified in the fiber. The released fiber from cocoon also indicates high biocompatibility that is promising as biocompatible suture.

Conclusion: The biocompatible fiber for future application as sutures is possible to be prepared by feeding the wild silkworm of A. atlas with leaf of E. variegata. The fiber is found rich with kalium (K) as well as chloride (Cl) with irregular shape of crystal at the surface of the fiber.

Keywords: Silk, herb, Erythrina variegata, Sutures, Biocompatibility.

INTRODUCTION

Recently, silk has been developed as engineering fiber to make artificial composite for biomaterial in medical application. Silks that are obtained from the cocoon are become long-standing interest by the scientist. The cocoon biophysics structure should be understood to make it constructive and effective. The scientist recently put attention on appearance and behavior of cocoon produced by different type of silkworm [1].

Silks are protein that is spun into fibers by larvae of lepidoptera such as silkworms, mites, scorpions, flies, and spiders. Silks are produced through biosynthesis process in epithelial cells. The proteins are stored into the lumen of these glands prior spinning into fibers. It exhibits mechanical properties to their specific functions and has a different composition of amino [2].

The silk moth Attacus atlas (Fig. 1) is an insect species that can produce silk. Not like domesticated mulberry silkworm of Bombyx mori that eat only one type of plant (mulberry plant) or monophagous, the larvae of A. atlas feed variety of plants (oligophagous). They often move from one plant to another in their development. The maximum wingspan of this moth reaches 300 mm. This is the largest wing of all moths. This species is found in Southeast Asia in tropical rainforest habitats at altitudes around 1500 m [3].

Tissues such as ligament, tendons, and bone are incapable of self-repair because of the diseased or damaged. It is required a substitute biomaterial to aid the healing process. Silk is slow degradability and biocompatible with impressive mechanical properties that render silk as a biomaterial for further exploitation. Silk fibroin protein is mechanically robust and biocompatible so it can be designed to desired specifications [4].

The larvae of silk moth spin the silk threads to form cocoons. The function of the cocoon is to enwrap for metamorphosis. The variations in climate and diet resulted diversity in the properties and types of the cocoons and fibers produced by silk within the same species. The cocoons are composed of two proteins, namely fibroin and sericin. The function of these protein structures includes protection from predators, pathogens, parasitoids, and assisting to complete metamorphosis by moisture and thermal. The larvae of A. atlas produce cuticular wax from abdominal tubercles. This wax forms a dense, white, powder coating over dorsal and lateral surfaces of the larva. The function of waxes is for conservation of the water, anti-parasitoid, and anti-predator [5].

Medicinal plants provide molecules that lead to the discovery of new drugs. Plants derived biologically active compounds have become important source of drug. Because of the recognition of herbal medicine as an alternative of health care, plants derived biologically active compounds have become important source of drug. Erythrina variegata plants contain phytopharmaceuticals, which have very important applications in the fields of medicine for the prevention of diseases. Plant of Erythrina variegata are phenolic, exhibit antiinflammatory, antimicrobial, antitumorogenic, anti-inflammatory, cardioprotective, and vasodilatory effects [6].
E. variegata has been used in folk medicine for the treatment of an antihelminthic and narcotic, venereal disease, toothache, asthma, insomnia, and malarial fever. Alkaloids from E. variegata were investigated for their anticancer activity, a muscle relaxant, as well as hemoerythrin [7].

The bark of E. variegata traditionally is used to treat stomachache and swellings. Its leaves are occasionally used to treat wind-damp obstruction syndrome in rheumatic joint pain and spasm of the limbs or lower back and knee pain, to stimulate lactation and menstruation for women and eye ailments. Isoflavones are compounds in plant foods, structurally similar to the mammalian estrogens recently received attention for use in the prevention of postmenopausal bone loss. Data from animal experiments provided evidence that isoflavones can attenuate menopausal bone loss, and it was suggested that isoflavones
In this research, the leaves from *E. variegate* are managed to become a food for *A. atlas* larvae. The cocoon obtained will be observed and the element contains in it will be identified as well as its biocompatibility to mammalian cells for future application as sutures.

**RESULTS AND DISCUSSION**

The egg of *A. atlas* was collected from their original location in Indonesia. The leaf of *E. variegate* (Fig. 2) was prepared from the source in Indonesia. Fig. 3 shows the appearance of caterpillars of *A. atlas* during consume leaf of *E. variegate*.

The special cage with the tree of *E. variegate* that was grown inside the cage was prepared. The caterpillars of *A. atlas* were let to consume the leaves *E. variegate* until reach their final stage inside the cage. At the final stage, the silkmoth provides themselves cocoon for final step of metamorphose.

The silk moth of *A. atlas* was let free exit from the cocoon for the next breeding. The cocoons left by the silkmoth were dried. The microstructure of the cocoon was investigated and recorded under scanning electron microscope (SEM). The energy dispersive X-ray spectroscopy method was introduced to reveal elements compositions in the cocoon. The result was presented in table and graphic.

For biocompatibility test, the cocoon of *A. atlas* was boiled in 0.1 M NaOH for about 1 h. Released fibers obtained were washed by hot water. Released fibrous tufts were sterilized with ethanol for 1 day at room temperature. Samples were washed by PBS. Fibrous tuft was soaked with suspension of U2OS cells in DMEM cultivation medium supplemented with 10% fetal bovine serum (105 cells per ml) for 2 h in the atmosphere of 5% CO<sub>2</sub>. The humidity was 95% and temperature at 37°C. The cell suspension was gently aspirated, and the fibers were immersed in the cultivation medium and the cells were cultivated for 2 days. The fibers were transferred to PBS and gently washed with PBS. Cells attached to the fibers were fixed with formaldehyde in PBS and washed. Nuclei were stained using DAPI (Sigma-Aldrich, USA) and actin microfilaments by phalloidin covalently conjugated with TRITC (Sigma-Aldrich, USA) in PBS. Fluorescence microscopy was carried by microscope IX-71 (Olympus, Japan).

**CONCLUSION**

The biocompatible fiber for future application as sutures is possible to be prepared by feeding the wild silkworm of *A. atlas* with leaf of *E. variegate*. The fiber is found rich with kalium (K) as well as chloride (Cl) with irregular shape of crystal at the surface of the fiber.

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