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Silkworm (*Bombyx mori*) and its constituents: A fascinating insect in science and research

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Abstract
Silkworm (*Bombyx mori*) is a highly domesticated and economically important insect which is the primary producer of silk. Apart from silk production *Bombyx mori* had a lot of applications in biological and scientific research. The present review focuses on the application of silkworm and its various constituents in science. Hemolymph which is an important body fluid is essential for regulation of the homeostasis constitutes plasma proteins, which function in various activities like maintaining cell cultures, defense against microbial infection and in anti-apoptosis. Sericin which is a silk protein has applications in pharmaceuticals and cosmetics. The chlorophyll derivative from silkworm excreta, could be used as a photo sensitizer for photo dynamic therapy of tumors.

Keywords: Silkworm, hemolymph, sericin, silkworm excreta

Introduction
Insects are a large, unexplored and unexploited source of potentially useful compounds for modern medicine [1]. Approximately 80% of animal species on earth are insects, 99% are invertebrates. We share a large proportion of our genetic material with all life on earth down to the simplest worms [2]. Silkworm (*Bombyx mori*) is one of the well-known beneficial lepidopteron insects for the production of sleek and sensuous silk fibre, often considered as "Queen of textiles". Infact the story of silk started almost five thousand years ago, while princess Xi Ling Shi was preparing a cup of tea, a silkworm cocoon from a mulberry tree fell into the hot water and its silk thread started unraveling. Silk is a highly versatile fabric which has proven to be ideal for a variety of uses from formal wear to sleep wear, from parachutes to rugs, from medical sutures to prosthetic arteries. In addition to its economic importance arising from applications in agribusiness, *Bombyx mori* is the main lepidopteron used in scientific research as a genetic resource capable of elucidating a wide range of biological problems [3]. The success of transgenesis of the silkworm has opened new prospects for this insect species [4].

Silkworm a model organism
The life cycle of *Bombyx mori* is very short and simple which includes four stages and considered one of the most advanced forms of metamorphosis. The various stages that it goes through during its life span are simply mesmerizing which includes embryo, larva, pupa and adult moth. The Silk worm is the larval stage of the silk moth's life cycle. Moths lay eggs which develop into larvae or caterpillar (commonly called Silkworms). They eat for 20-30 days, consuming large amounts of mulberry leaves and moult through five changes of skin or 'instars. In almost 2 days a silkworm spins a single thread that is about 1,000 to 3000 feet long which is the pupal stage. Finally the adult Silk moth emerges out of the cocoon and female deposits around 400 eggs at a time.

Apart from being a great economic insect in sericulture all over the world, the silkworm also proved its importance in biotechnology as a bioreactor for the production of recombinant proteins and silk-based biomaterials [5, 6]. In the development of drugs also silkworms have more advantages over mammals regarding the expenses and ethical issues since the body size is large enough for hemolymph preparations and organ isolation, which are essential for studying the pharmacodynamics of drugs in animal bodies (Fig 1). There is similarity between the humans and silkworms regarding the lethal effects observed during infection with pathogenic microorganisms and as well as in therapeutic strategies used in the form of antibiotics against those organisms [7] along with the effective doses (ED50) of antibiotics in both human and silkworm infection models [7,8].

The insect fat body is an organ that functions in drug metabolism, similar to the liver in mammals where a number of enzymes such as sulfotransferases which are involved in drug detoxification, are present in the fat body [9]. Hence Silkworms have been used as infection models to screen and to discover novel therapeutic antimicrobial agents [7, 10] and evaluating the pathogenicity of bacteria and fungi [11, 12]. Studies also indicate that the LD50 values of cytotoxic chemicals in silkworms are consistent with those in mammals, suggesting that common pathways are used for chemical metabolism between silkworms and mammals [13]. The hyperlipidemic silkworm model was established which are useful for evaluating the therapeutic activities of anti-diabetic drugs regarded as a model of type 2 diabetes [14]. Coming to the genome organization of *Bombyx mori*, it was the first organism in the order lepidoptera for which a draft of the genomic sequence [15,16] were completed with the haploid nuclear genome of size 530 mb which is broken into 28 chromosomes [17].

Silkworm hemolymph

Silkworm hemolymph prepared from *Bombyx mori* is an insect serum that has been widely used as a medium supplement in insect or animal cell cultures because it can be prepared easily and economically [18]. Recently, SH has been used as a medium supplement in animal cell cultures for three purposes: for the prevention of apoptosis [19] for the improved production of recombinant proteins [20], and for the stimulation of cell growth. Silkworm hemolymph inhibits Baculovirus-induced insect cell apoptosis [21] and during this infection, highest anti apoptotic activity was exhibited by a non-glycosylated monomeric protein with molecular weight of Ca. 28,000 Da protein [22]. Silkworm hemolymph inhibits apoptosis not only in the insect cell system but also in the human cell system. Hence silkworm hemolymph can be effectively used to minimize cell death in commercial animal cell cultures [23]. 30K protein of silkworm hemolymph have been reported to exhibit anti-apoptotic activity in various mammalian and insect cell systems and could have therapeutic potential in diseases related to apoptosis [24]. It is well documented that insect hemolymph contains many antimicrobial substances, like melanin, immune proteins and antimicrobial Peptides (AMPs) to protect against invading pathogens as they do not have any adaptive immune system. AMPs are used in novel therapeutics development for other diseases, such as Atopic dermatitis, Crohn's disease, Cystic fibrosis [25] and tumours [26]. Earlier reported, that silkworm *Bombyx mori* produces antibacterial protein Attacin (~20 kDa) and Gloverin (~14 kDa) have abilities to kill *E. coli* [27]. Many antibacterial peptides were isolated from silkworm hemolymph such as Moricin [28], Lebocin which is a first proline rich anti-bacterial peptide found in lepidopteran insect [28].

Moricin functions as an antibacterial peptide against gram-positive and negative bacteria and inhibit growth of the bacteria by binding to DNA gyrase, glycerol phosphate lipoteichoic acid synthase and dipeptide ABC transporter of bacteria [29]. Therefore these can be used against multi drug resistant (MDR) pathogens. The studies on brain factor (BF-7), a natural component in silkworm indicates the learning and memory enhancing activity i.e., by the production of neurotransmitter acetylcholine. Thus BF-7 could be an excellent cognitive enhancing drug.

Silk protein sericin

Silk worm cocoons contain two major protein components, fibroin and sericin (Fig 2). Sericin is a glue protein which keeps two fibroin filaments together. Fibroin is used in textiles, industrial and medical applications where as sericin is usually discarded as waste in textile industries during separation of fibroin filaments from sericin. It is estimated that each year 50,000 tons of unutilized sericin was discarded in degumming wastewater worldwide. The recovery and reuse of sericin usually discarded by the textile industry not only minimizes environmental issues but also has a high scientific and commercial value. The physicochemical properties of the molecule are responsible for numerous applications in biomedicine. Silk fibroin can be used as the substratum for the culture of animal cells in place of collagen [30]. Fibroin supports cell attachment and proliferation for a variety of cell types [31, 32].

In vitro and *in vivo* studies of biocompatibility and antioxidant potential, demonstrated that sericin is immunologically inert and also have proven the safety and open the wide possibility of applications of sericin in biomedicine, such as the food and cosmetic industries and in tissue engineering [33,34]. Pharmacological and biomedical applications of sericin includes antioxidant potential [35], anti-cancer activities [36], anti-coagulant [37], liver and gastric protection [38], tyrosinase inhibition and stimulate immune system [39] and as well as cholesterol lowering [40]. It has also been proven that silk protein sericin has a curative effect in diabetes [41] and very high wound healing property [42]. In cell cultures, sericin stimulates mitosis [43], promotes cell proliferation [44], protects against cell death [45]. Hence sericin can also be used instead of FBS in the freezing media, for different cell types such as human adipose tissue-derived stem cells [46], myeloma cell lines, ovarian cells, fibroblasts, keratinocytes and insect cell lines [47], rat insulinoma cell lines, and mouse hybridoma cell lines [48]. Intake of sericin containing food relieves constipation, suppresses the development of bowel cancer and accelerates the absorption of minerals. Sericin when taken orally causes a dose dependent decrease in the development of colonic aberrant crypt foci [49]. Silk sericin has the potential to find application in the development of contact lenses. Oxygen permeable membranes are made up of fibroin and sericin with 10-16 percent water and are used for contact lenses and as artificial skin [50]. Ethanol extract of the sericin layer of cocoon shells provide a novel stock which, together with sericin protein, has potential uses in functional food, biotechnological and medical applications [51].

Silkworm litter

Silk worm litter is a waste from rearing trays (Fig 3). The sericulture waste which includes larval excreta, leaf litter, dead larvae, moth and cocoons contain organic matter [52]. Seri waste compost contains approximately 2.00-2.24% N, 0.93-1.00% P and 1.5-1.8% K besides Zn, Fe, Mn and Cu as micronutrient [53]. The Silkworm litter is presently used as fodder and as compost and the pupal waste is utilized in oil extraction, biogas production, mushroom cultivation [54]. The application of compost manure produced out of sericulture waste including silkworm litter is highly beneficial for mulberry cultivation and is much effective than conventional use of farm yard manure [55]. Silkworm litter could be used as a nitrogen source in replacing chemical fertilisers for vegetable crop production and as a substrate for mushroom cultivation. Since the pupae contains high amount of nitrogen

and protein, there is potential for the bio conversion of pupal waste to enriched compost and utilization as a nutrient source [56]. Silkworm litter and pupal waste also used for mass production of *Bacillus thuringiensis* [57]. *B. thuringiensis* is the most successful commercial biopesticide accounting for 90% of all biopesticides sold all over the world. Apart from these uses, Chlorophyll derivatives (CpD-A, -B, -C, and -D) were extracted from Silk worm (*Bombyx mori*) excreta, among them CpD-A was extensively studied to clarify its role as a "photosensitizer" for photodynamic therapy (PDT) of tumors *in vitro*. These findings suggested that CpD-A produced by use of silkworm excreta could be used as a photosensitizer for PDT of tumors by the use of lights of near 650 nm [58].

Fig 1: Fifth instar silkworm larvae.

Fig 2: Silkworm cocoons.

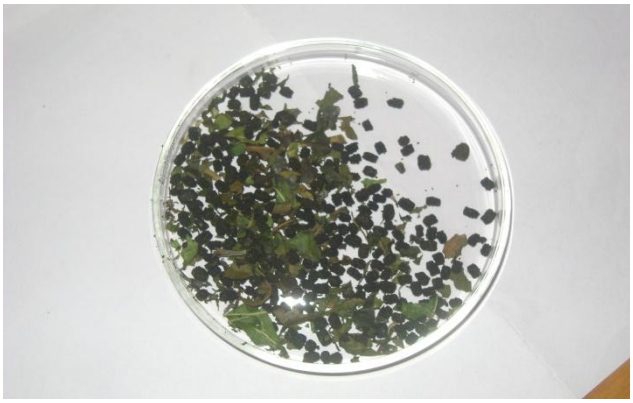

Fig 3: Freshly collected silkworm litter.

Conclusion

In spite of its appearance, silkworm takes after human in lots of ways such as analogous tissues or organs, similar sensitivities to pathogens and comparable effects of drugs and it is low in cost, in a little conflict with ethical problem and in no danger of biohazard. Therefore, silkworm is an excellent tool for drug screening and safety test. Rapid emergence of (MDR) multi drug resistant bacteria is a burning threat to human health worldwide; it raises the urge to develop some alternative compound, which would be effective against MDR pathogens. Here silkworm hemolymph takes the place to fight against MDR pathogens as it is rich Source of many antimicrobial peptides and as well as anti-apoptotic components. Sericin which is a silk protein has applications in pharmaceuticals and Cosmetics such as wound healing, bio-adhesive, moisturizing, anti-wrinkle and anti-aging. Seri waste compost contains high amount of nitrogen and protein, thereby effective utilization of sericulture waste minimize the environmental pollution, reduce the cost of inorganic fertilizers uses and a good alternative to restrict the use of inorganic fertilizers. Apart from these derivatives from silkworm litter could be used for therapeutics for tumors.

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