REVIEW ARTICLE

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Silkworm in Pharmacology and Toxicology

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Abstract

For a long and healthy life, new drugs, medical services, and care must be developed against diseases. Laboratory animals are used in pharmacology and toxicology studies. The use of laboratory animals causes serious problems such as animal welfare and cost. Cell culture, which constitutes the first step of drug development, is used to evaluate the safety and effectiveness of drugs; however, it can cause failure due to unpredictable liver toxicity and bioavailability problems. Invertebrates are used to determine the desired effect in the early stages of drug development. Silkworm, an invertebrate, is one of the best models to represent genetic, biochemical studies due to its complex metabolism, large body, the abundance of mutants. Silkworms have been used in many studies on pathogenic microorganisms in the world. The silkworm infection model and therapeutic efficacy of antiviral, antifungal, and antimicrobial agents (ED_{50}) are consistent with the mammalian model. Silkworms have cytochrome P450s and conjugating enzymes (glutathione S-transferases, UDP-glucosyltransferase, ubiquitin-conjugating enzyme E2 etc.). Silkworms can be used in detecting tissue damage caused by drugs and chemicals, in antidiabetic drugs, and herbal studies. Silk beetles are used to investigate the treatment of diseases, but cannot fulfill the basic obligations of pathological study. In this review, the information will be given on the use of silkworms as a pharmacological and toxicological animal model.

Keywords: Animal model, pharmacology, silkworm, toxicology.

INTRODUCTION

Silkworm *Bombyx mori* has been tamed to produce silk for nearly 5000 years. Due to its complex metabolism, large body, the abundance of mutants, silkworm in the *Lepidoptera* team is one of the best models to represent genetic and biochemical studies (Nagaraju and Goldsmith, 2002; Goldsmith et al., 2005).

Silkworm's egg, larvae, pupae, butterfly forms, and their products' are used as animal drug sources. Amino acids, adipokinetic hormone, beta N-acetylglucosaminidase, sex pheromone bombykol, chymotrypsin inhibitors, etc., found in the form of larvae. The substances are used in the treatment of trigeminal neuralgia, vocal nodules, and polyps, facial paralysis, and pain. Substances such as vitamins B1, B2, and E, diapause hormone, proteins, and amino acids in the form of pupa fall into the structure of antibacterial and antihistamine drug preparations. The male butterfly form is used for sterility treatment. Extract of silkworm feces contains pectin, chlorophyll, carotene, phytol, and triacontanol, solanesol, etc. is used in various diseases such as leukocytopenia, acute pancreatitis, hepatitis, chronic nephritis, stomach ailments; Besides, these substances have the effect of reducing cholesterol and blood sugar. Since silkworm products contain stearic, palmitic, linoleic acids, they are used in pharmaceutical preparations and as food additives (Singh and Jayasomu, 2002).

For people who want to live a long and healthy life, new drugs, medical services, and care must be developed against diseases. Laboratory animals (mice, rats, rabbits) are used in medicine, oncology, toxicology, mycology, tissue and organ culture, immunology, and reproductive studies (Sarıözkan, 2005). To evaluate the results correctly in the pharmaceutical and food production stages, the sacrifice of many animals such as mice and rats causes serious problems such as animal welfare and cost.

In the text of Russell and Burch's Principles of Human Experiment Technique in 1959, it was stated that there should be alternative methods of reduction, replacement, and refinement in laboratory animals with the 3R rule (Russell and Burch, 1959). Sensitivity to animal welfare in the world has been affecting the drug development sector negatively in recent years (Sekimizu and Hamamoto, 2016).

Cell culture, which constitutes the first step of drug development steps, is an expensive method used to evaluate the safety and effectiveness of therapeutic drugs before preclinical animal studies (Mazzoleni, 2009; Breslin and O'Driscoll, 2013). Metabolism and toxicity costs of cell culture, deficient pharmacokinetics, loss of drug-metabolizing enzymes in long-term culture causes drug development failure. Many of the substances studied from in vitro cultured cell systems have no therapeutic effect since pharmacodynamics cannot be determined in the target animal (Sivaraman et al., 2005; Hopkins, 2008; Sekimizu and Hamamoto, 2016). Mammalian models are used in pre-clinical animal studies from drug development stages. Collecting information by working on a large number of mammals in preclinical studies causes financial and ethical problems (Orlans et al., 1999). Invertebrate animals can be used in the early stages of drug development to solve these problems (Breger et al., 2007; Sekimizu and Hamamoto, 2016)

Nematodes used as invertebrates (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*) honeycomb moth (*Galleria mellonella*) are very small in the evaluation of therapeutic effects, causing problems in the evaluation of the results due to the injection area and

the sample volume to be taken (Needham et al., 2004; Mylonakis, 2005; Breger et al., 2007). Silk beetles, one of the invertebrate models, have been used as models for infection in human pathogenic bacteria (Kaito et al., 2002; Hamamoto et al., 2004). Silkworm has been used as a model for a long time due to the ease of growing in the laboratory, small growing area, body size, and contribution to the economy. (Fujii et al., 1998). With the silkworm infection model, the pharmacology and toxicology of the compounds investigated in the studies are also evaluated (Hamamoto et al., 2009). A large number of silkworms can be produced with artificial food at any time of the year at a low cost. It has been determined that some antibiotics of median effective dose (ED_{50}) values are compatible with mammalian animal models in silkworm infected with human pathogen infectious agents (Kaito et al., 2002; Hamamoto et al., 2004; Hamamoto and Sekimizu, 2005).

In silkworm diseases, Flacherie (*Escherichia coli, Streptococcus, Bacillus, Proteus sp* and *Staphylococcus*), Muscardine (*Beaueriana bassiana, Aspergillus flavus, A. oryzae, A. tanei, Paecilomyces farinosus, Sporosporella uvella, Metarhizium anisophia*), *Nosema spp., nuclear polyhedrosis* virus, *cytoplasmic polyhedrosis* virus and *densonucleosis* virus infect silkworm (Fujıwara, 1980; Watanabe, 2002; Kumar et al., 2009). *Microsporidia* are commonly found in nature that infects all vertebral and non-vertebral hosts. It also infects silkworms. It causes diarrhea in humans (*Encephalitozoon species*) (Kaya et al., 2008).

In the laboratory, rabbits, mice, and rats can accommodate many viral, parasitic, fungal, and bacterial agents. Researchers should be aware of the effects of these agents on studies. The reliability of a scientific study also requires laboratory animals to be free from viral, bacterial, and fungal diseases (Müftüoğlu and Albayrak, 2019). In mice rats and rabbits Mousepox, Lymphocytic Choriomeningitis Virus, Minute Virus, Adenovirus, Mouse Cytomegalovirus, Mouse Hepatitisvirus, Rotavirus, Reovirus Type 3, Sendai Virus, Encephalomyelitis virus, Kilman Rat Virus, Toolan H-1 Virus, Rat Coronavirus, Hemorrhagic Syndrome Virus, *Klebsiella pneumoniae, Mycoplasma pulmonis, Streptococcus pneumoniae, Helicobacter spp, Pseudomonas aeruginosa, Staphylococcus aureus, Bordetella bronchiseptica, Clostridium spiroforme, Staphylococcus aureus*, Intestinal coccidiosis, *Sarcoptes scabiei* agents cause disease. Such animals may cause lots of diseases such as Leptospirosis, Listeriosis, Pseudotuberculosis, Salmonellosis, Toxoplasmosis, Rat Bite Fever, Tularemia, Tuberculosis, Tyzzer Disease, Hantavirus Infection, Lymphocytic Choriomeningitis Pasteurellosis, Triposomiasis, Dermatophytosis via direct contact, respiration, fecal-oral, bite wounds and indirect ways (Baker, 1998; Gül et al., 2013).

Silkworms have cytochrome P450s and conjugating enzymes (glutathione S-transferases, UDP-glucosyltransferase, ubiquitin-conjugating enzyme E2 etc.) (Luque et al., 2002; Hamamoto et al., 2005; Li et al., 2005; Gao et al., 2006; Yamamoto and Yamada; 2016). Drug injection is provided in silk beetles through the midgut and hemolymph (Figure 1). Many pathogens have been studied in the world with silkworms. With the silkworm infection model, the therapeutic effectiveness of antiviral agents used in viral diseases of humans has been tested in silkworm compatibility. In the study with Baculovirus, the amount of maximal inhibitory concentration (IC_{50}) of some antiviral drugs required to inhibit the ED_{50} and the virus was determined (Figure 2). It was stated that the amount of antiviral agents used at the end of the study is consistent with the amount used in humans (Kool et al., 1995; Szewczyk et al., 2006; Orihara et al., 2008).

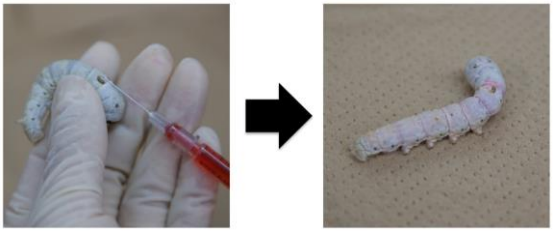

Figure 1. Drug administration to silkworm hemolymph (Hamamoto and Sekimizu, 2016).

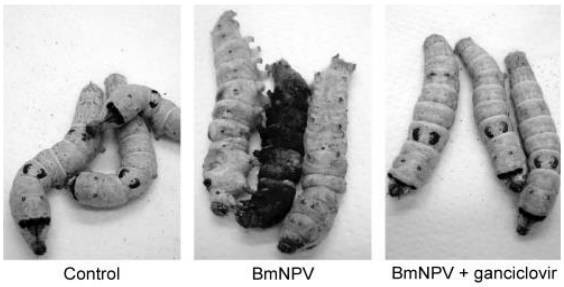

Figure 2. Baculovirus infection model and the effect of the antiviral agent (Orihara et al., 2008).

Cryptococcus neoformans, a pathogenic fungus in humans, is caused by cryptococcosis. As an invertebrate animal, *Bombyx mori*, nematodes (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*), honeycomb moth (*Galleria mellonella*), pathogen in humans in Mus musculus animals *Cryptococcus neoformans* comparison was made as a result, silkworm's body size, human body temperature 37 Cº survival. It has been stated that injection can be used instead of mice and rats in order to create *Cryptococcus neoformans* infection due to the injection of two ways, namely hemolymph and appropriate sample taking (Ishii et al., 2016). The therapeutic effects of antifungal drugs, amphotericin B, flucytosine, fluconazole, and ketoconazole were determined in animals that had *C. neoformans* infection in silkworm. As a result, it was emphasized that to evaluate the therapeutic effects (ED_{50}) of antifungal drugs, each drug can be studied *in vivo* toxicity and pharmacokinetics, and results in matching mammalian infection models were obtained (Matsumoto et al., 2012).

In silkworms, hyperglycemia can be created with foods containing high glucose (Figure 3). Extract of Chinese foxglove (*Rehmanniae Radix)*, a hypoglycemic effect was shown in the study conducted with the hyperglycemic silkworm model (Figure 4). It has been stated that it can be used in anti-diabetic drug studies for type I and type II diabetes with the silkworm model. As a result, it has been stated that silkworm can be used in herbal studies (Matsumoto and Sekimizu, 2016).

Silkworm larvae are sensitive to some human pathogens (*Pseudomonas aeruginosa, Candida albicans, Staphylococcus aureus, Vibrio cholerae*) and even cause their death. The application of antimicrobial or antifungal agents in the treatment of these diseases prevents the silkworm from killing these pathogens (Kaito et al.,2002;

Hamamoto et al., 2004; Nwibo et al., 2015; Uchida et al., 2016). Lysocin E, which was discovered as a novel antibiotic, was determined with its efficacy (ED_{50} and MIC values) against *S. aureus* by a silkworm infection model (Table 1) (Hamamoto and Sekimizu, 2016). A natural antifungal compound has been reported in a study conducted on silkworm larvae infected with *Aspergillus fumigatus* (Nakamura et al., 2017). In a study, it was stated that silkworm larvae can be used as a human infection model in treatment and drug development studies in Parkinson's disease (Tabunoki et al., 2016). It has been declared that transgenic silkworms can be used as a bioresource in the development of therapeutic glycoproteins in diseases such as Lysosomal storage disease (LSDs) (Itoh et al., 2016). It has been announced that the administration of drugs such as hepatotoxic chemicals to silkworms can be used as an alternative animal model in the evaluation of drug-induced tissue damage (Figure 5) (Inagaki et al., 2016).

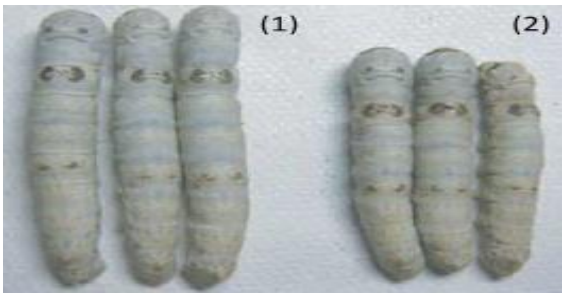

Figure 3. Hyperglycemic silkworms model normal diet (1) and glucose solution (2) (Matsumoto et al., 2011).

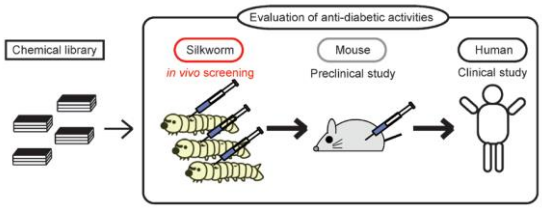

Figure 4. Anti-diabetic drug studies with silkworm model (Matsumoto and Sekimizu, 2016).

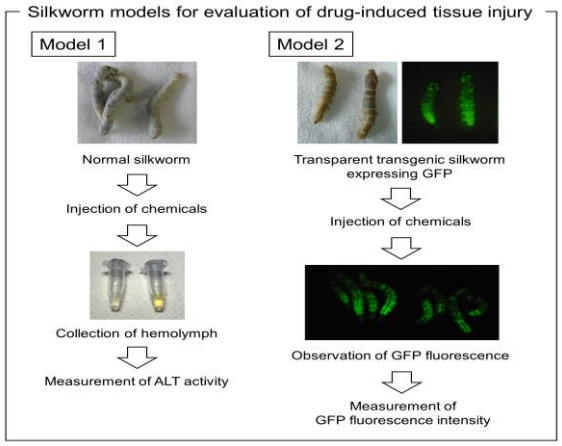

Figure 5. Determination of tissue damage using the silkworm model (Inagaki et al., 2016).

It has been stated that silkworms can be used in drug development instead of mammals in the future. However, silkworms cannot be used for genetic diseases such as sensory and neurological disorders. It cannot fulfill the basic obligations of the pathological study. Although it does not meet the expectations of the mammalian model, it has a complementary ability (Guo-Ping and Xi-Jie, 2011; Meng et al., 2017). As a result, we think that silkworms can be used as an experimental animal model in pharmacological and toxicological studies. More studies are needed in this direction.

Table 1. Correspondence of silkworm infection model and therapeutic efficacy of antibiotics against *S. aureus* with ED_{50} values in the mouse model (Hamamoto and Sekimizu, 2016).

Drugs	**ED_{50} (mg/kg- animal)**	
	Silkworm	**Mouse**
Teicoplanin	0.3	0.1
Vancomycin	0.3	1
Linezolid	9	4
Katanosin B	0.1	0.7
Flomoxef	0.2	0.3
Minocycline	4	1

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Conflict of Interest

The authors declare that there is no conflict of interest in the content of the article

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