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Research Article

SCREENING OF ORGANIC SOLVENTS IN AN SILKWORM, AN INVERTEBRATE ANIMAL MODEL

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ABSTRACT

More than 40% NCEs (new chemical entities) developed in pharmaceutical industry are practically insoluble in water, thus organic solvents are required to solubilise the drugs. But, the solvents interfere with the biological activity of the test drug and results in the toxicity. Therefore, the suitability of the solvent needs to be accessed for its toxicity. So far, the mammalian models have been used for screening the therapeutics drugs. But using the mammalian models involves problems interms of cost and moral issues. Hence, to overcome these problems we suggest silkworm as a model to evaluate the toxicity and the therapeutic effects of the chemicals. Hence, in the present study we used organic solvents like Ethanol, Acetonitrile, and DMSO to examine the toxicity of the solvents in the silkworm. The solvents were injected into the silkworm haemolymph and the preliminary tests were conducted to find out the median lethal dose (LD_{50}) of the silkworm for 24h by probit analysis method. The LD_{50} values of ethanol, acetonitrile, DMSO is 10 µl/g, 1.99 µl/g, 19.95 µl/g. Our experiment successfully demonstrated that silkworm can be used as a model organism for evaluating the toxicity of solvents.

INTRODUCTION

In the past few decades, the mammalian models have been used for screening and evaluating the therapeutics drugs. But using the mammalian models involves problems interms of cost, maintenance, difficult to maintain pathogen free facilities and ethical issues which is regulated by various agencies across the world (Levy, 2012; Kaito *et al*., 2007). Thus it is difficult to use the mammalian models because of modern animal rights and animal welfare laws. In order to overcome the problems associated with the modern animal rights, a mammalian model can be replaced with an invertebrate animal model, so that it can reduce the rate of mortality of the mammals (Renwick *et al* ., 2007). Hence we suggest silkworm as a model to evaluate the toxicity and the therapeutic effects of the chemicals (Suresh *et al*., 2017).

Silkworm has recently emerged as an ideal model for *in vivo* studies using small molecules. It gained importance in the field of biotechnology for the production of recombinant proteins (Altman, 2003; Kato *et al*., 2003) and life science (Nwibo *et al*., 2015), it has advantages over mammals like their use of as an experimental model is less complicated, does not require ethical clearance (Chen *et al*., 2014), less cost as compared to vertebrates. From the anatomic perspective, silkworm harbours most of the organs such as gut, fat body, malphigian tubules, correspond to the intestine, liver and kidney respectively in the mammals (Yamato *et al*., 2005). The silkworm shares monotonous mechanism of drug metabolism with mammals. In particular, chemicals consolidated in the silkworm body are altered by hydroxylation of the first stage reactions by cytochrome P450s proceed by the second stage reactions of conjugation to exceedingly water-soluble substances are metabolised by excretion. The findings of Hamamoto (2009), additionally found that a model compound is processed in silkworms by the cytochrome P450 protein, takes after the metabolic pathway through the conjugation response, and shows the comparative pharmacokinetics as in vertebrates (Hamamoto *et al*., 2009). With these unique features, Silkworm can be used as an animal model organism to elucidate the toxicity of the compounds and various processes in life sciences (Suresh *et al*., 2017).

Over 40% NCEs (new chemical entities) developed in pharmaceutical industry are practically insoluble in water. As many compounds or drugs exhibit limited solubility in aqueous solution, thus the use of organic solvents is required to solubilise the drugs. Solvents are chemical substances that can

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dissolve, suspend or extract other materials usually without chemically changing either the solvents or the other materials. They frequently play a role at the start of the pharmaceutical manufacturing process. Solvents work in an assortment of approaches to contribute to many of the medicines today, As helpers in the formulation of many health care products such as penicillin, aspirin, cough syrup, and topical ointments. But, the use of solvent results in the toxicity and may interfere with the biological activity of the test drug (Forman *et al*., 1999). Therefore, the suitability of the solvent needs to be evaluated for its toxicity.

Hence, we used organic solvents like Ethanol, Acetonitrile, Isoproponal, Acetone, Methanol and DMSO to examine the toxicity of the compound in the silkworm. Toxicity evaluation of a compound will be highly useful in the final evaluation of designing 'safe level or tolerable level' of compound. Silkworm exhibit the 50% of the lethal doses (LD_{50}) of the chemicals as in the mammals (Usui *et al*., 2016; Hamamoto *et al*., 2009). LD_{50}, a typical measure of toxicity is the lethal concentration that causes death in 50% of the treated mammals. LD_{50} predominately expressed as the dose in milligram (mg) of chemical per kilogram (kg) of body weight, LD_{50} is usually expressed as milligram of chemical per volume of medium. The model is exposed to chemicals are considered exceedingly toxic when the LD_{50} is less and essentially non-toxic when the esteem is expansive.

For evaluating, toxicity of a substance there are diverse parameters, for example, lethal, sub-lethal dosage, safe measurements and so forth. The toxicity can be assessed by injecting the silkworms to various dosages for settled time and after reasonable interims score the number of animals dead (or) alive (Finney, 1971). The toxic quality relies upon factors like regularity and body estimate, its developmental stage, exposure time, sex, age, dietary status and natural parameters and so forth. A few reports are accessible on lethality assessment of concentration of the substances on different insects including *B. Mori* (Singh *et al*., 1984; Srivastava *et al*., 1985; Christian *et al*., 1986; Thay *et al*., 1986; Venkata *et al*., 1989; Matsumoto *et al*., 2012; Hamamoto *et al*., 2009). LD_{50} Values are important to evaluating the toxicity level and also to determine the sub-lethal doses. The present study is helpful to evaluate the impact of solvents and behavioral changes in silkworm on inducing acute and sub-acute doses of solvents.

MATERIALS AND METHODS

Animals

Silkworm *Bombyx mori*., L ($CSR_2X\ CSR_2$) of fifth instar with an average weight of 1.5 gm were acclimatized to laboratory under standard rearing conditions temperature of 24.0±1°C and humidity 75% ±1 % .

Reagents

Ethanol, Acetonitrile, DMSO were obtained from the Hi-media Bioscience Bangalore.

Administration of solvents in silkworm haemolymph

The solvents are injected into the silkworm haemolymph for the toxicity study. Preliminary tests were conducted to find out the median lethal dose (LD_{50}) of the silkworm for 24 h by probit analysis method (Finney, D.J., 1971).

RESULTS

In present study, three solvents were chosen for the toxicity analysis in silkworm larvae. The solvents included ethanol, acetonitrile and DMSO. For this study, the effect of a 24 hour exposure to each solvent was analysed.

Ethanol

The silkworm exposed to different doses of ethanol exhibited 10% mortality at 2 µl/g., 30% mortality at 5 µl/g., 50% mortality at 10 µl/g., 60% mortality at 15 µl/g., 100% mortality at. 20 µl /g, The results are presented in the table 1. The probit was plotted against log dose of the ethanol, showed straight line (Fig 1). The 24 h LD_{50} value obtained from straight line graph is 10 µl/g.

Table 1 Probit Mortality analysis of different doses of ethanol at 24 hr silkworm, *Bombyxmori*

Sl.No	Concentration	Log Dose	Total Larvae	No of Dead Larvae	% Mortality	Probality
1	0	0.00	10	0	0	-
2	2	0.301	10	1	10	3.72
3	5	0.699	10	3	30	4.48
4	10	1.000	10	5	50	5.00
5	15	1.176	10	6	60	5.25
6	20	1.301	10	10	100	-

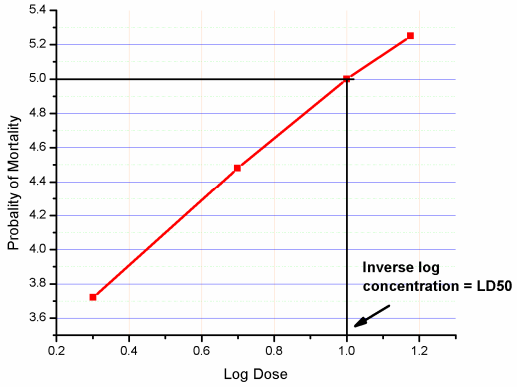

Figure 1 Plot of log-doses versus probits of ethanol for calculation of LD_{50}

Acetonitrile

Table 2 represents the results of the silkworm exposed to different doses of acetonitrile exhibited 50% mortality at 2 µl/g., 70% mortality at 50 µl/g., 80% mortality at 10 µl/g., 90% mortality at 15µl/g and 100% mortality at 20 µl/g. The probit was plotted against log dose of the acetonitrile, showed straight line (Fig 2). The 24 h LD50 value obtained from straight line graph is 1.99µl/g.

Table 2 Probit Mortality analysis of different doses of Acetonitrile at 24 hr silkworm, *Bombyxmori.*

Sl.No	Concentration	Log Dose	Total Larvae	No of Dead Larvae	% Mortality	Probality
1	0	0.00	10	0	0	-
2	2	0.301	10	5	50	5.00
3	5	0.699	10	7	70	5.52
4	10	1.000	10	8	80	5.84
5	15	1.176	10	9	90	6.28
6	20	1.301	10	10	100	-

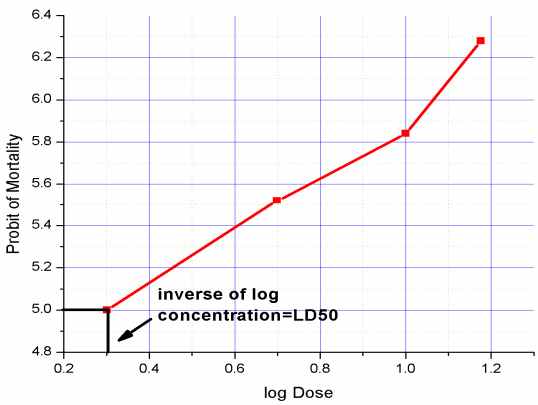

Figure 2 Plot of log-doses versus probits of acetonitrile for calculation of LD_{50}

DMSO

Table 3 represents the results of the silkworm exposed to different doses of DMSO exhibited 10% mortality at 5µl/g ., 20% mortality at 10µl/g., 40% mortality at 15µl/g and 50% mortality at 20µl/g. The probit was plotted against log dose of the DMSO, showed straight line (Fig 3). The 24 h LD50 value obtained from straight line graph is 19.95µl/g.

Table 3 Probit Mortality analysis of different doses of DMSO at 24 hr silkworm, *Bombyxmori.*

Sl.No	Concentration	Log Dose	Total Larvae	No of Dead Larvae	% Mortality	Probality
1	0	0.00	10	0	0	-
2	2	0.301	10	0	0	-
3	5	0.699	10	1	10	3.72
4	10	1.000	10	2	20	4.16
5	15	1.176	10	4	40	4.75
6	20	1.301	10	5	50	5

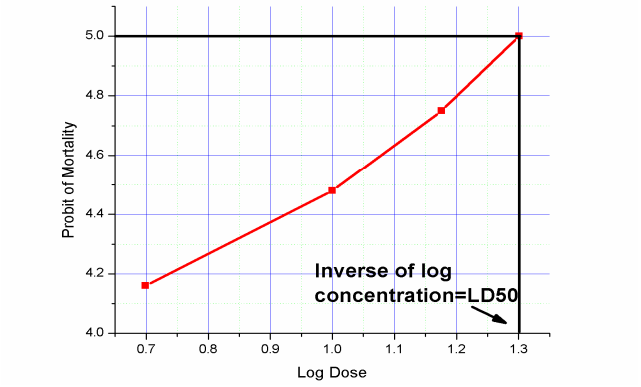

Figure 3 Plot of log-doses versus probits of DMSO for calculation of LD_{50}

DISCUSSION

Traditionally mice is used for screening, identifying and assessing the drugs, but according to the animal welfare laws, sacrificing an extensive number of well evolved mammalian models for evaluation of the compounds brings about not just a high cost yet can likewise raise moral issues with respect to animal welfare. To tackle these issues, we are proposing utilization of the silkworm as an animal model (Kaito and Sekimizu, 2007; Renwick and Kavanagh, 2007; Altman *et al*., 2003). The silkworm is an insect whose reproducing strategy has been well developed during a long history of sericulture. Silkworm model is portrayed by its convenience, minimal effort, and the utilization of silkworms keeps away from ethical issues that emerge when vertebrates are utilized for research. Therefore, we can easily conduct experiments using a large number of silkworms. In addition, utilizing silkworms have many advantages like they move gradually furthermore, their size is bigger in contrast with other invertebrate animal models, like fruit fly or nematode. By using syringes, one can infuse exact volumes of tests containing pathogens or antibiotics into the haemolymph of the silkworms. Moreover, the fat body is identified with the drug metabolism like the liver in mammals. The gut is the principle extensive organ of larvae related to absorption, metabolism and transportation of different substances. Those tissues have imperative roles in the pharmacokinetics in silkworm as well as in mammals (Matsumoto *et al*., 2012).The similarity of immune response and comparative pharmacokinetics of silkworm contrasted with vertebrates. Silkworms are considered as an appropriate animal model for evaluating therapeutic effects of chemicals (Hammato *et al*., 2004; Kaito *et al*., 2004; Ishi *et al*., 2008).

Furthermore, the LD_{50} value, the amount of reagents required to kill 50% of animals, of chemicals against silkworms resemble well with that in mammals (Usui *et al*., 2016; Hammato *et al*., 2009) and thusly quantitative assessment of both the toxic and therapeutic impacts of compounds can be a simultaneously performed with the silkworms. Together, these discoveries show that the fundamental pharmacokinetic features of chemicals are basically comparative amongst silkworms and mammals. Thus, Silkworm is recommended to be an appropriate and useful animal for screening drug candidates that have therapeutic effects without toxicity in mammals (Hamamoto *et al*., 2015).

This study was designed to identify toxicity of the solvents which are already widely used for dissolving drugs, that might be potentially influencing pharmacological or toxicological screens. The toxicity evaluation of the solvents in the silkworm was performed to find the safe level or the tolerable level of the solvents. The Preliminary tests were conducted to find out the median lethaldose (LD_{50}) of the silkworm for 24 h by probit analysis method (Finney, 1971). The minimum dose at which the zero percent mortality and the maximum dose at 100% was studied. The LD_{50} values were calculated for the solvents as listed in materials and methods as shown in figure 1-3.

The typical symptoms of exposure to solvents intoxication were observed, the silkworm in the control group were actively feeding and developments were well co-composed. The silkworm exposures to the dosages of solvents ended up irritable and hyper energized developments, unusual slithering developments were noticed. At high dosage of the solvents, silkworms gradually were turned to be noticeably lazy with sharp jerky developments. In addition, silkworm stopped eating and their body became stiff and blackened followed by outflow of black liquid. At long last the silkworm settles down at some place with the loss of balance and resulted in death.

The LD_{50} values of ethanol, acetonitrile, DMSO were 10 µl/g, 1.99 µl/g, 19.95 µl/g respectively. The DMSO was less toxic when compared with the other solvents (table 4). These results support the findings of the Jan Maes (2011). Our experiment successfully demonstrated that silkworm can be used as a model organism for evaluating the toxicity of solvents. The

knowledge of toxicity studies and behavioural observations are useful to establish limits and levels of susceptibility of solvents .Thus Silkworm stand out to be a valuable model in field of toxicology for drug designing at safer level.

CONCLUSION

Solvents are chemical substances that can dissolve, suspend or extract other materials usually without chemically changing either the solvents or the other materials. They play an important role toward thebegin of the pharmaceutical manufacturing process. Solvents work in an assortment of approaches to contribute to many of the medicines today. The use of solvent results in the toxicity and may interfere with the biological activity of the test drug. Therefore, the suitability of the solvent needs to be evaluated for its toxicity. Toxicity evaluation of a compound will be highly useful in the final evaluation of designing 'safe level or tolerable level'of compound. Thus, the study was designed out to identify the toxicity of the solvents using silkworm as an animal model. As, Silkworm which offer technical advantages in drug development and use in the pharmaceutical-biomedical research. In addition, silkworms can be used as a primary screening system that has several advantages, including an established rearing system, cost effectiveness, reproducible and robust application, no moral issues, and conserved metabolic pathways with mammals.

Compliance with Ethical Standards

Conflict of interest

Authors do not have any conflict of interest related to the manuscript.

Ethical approval

Silkworm does not require any ethical approval. As Silkworm is an invertebrate animal model.

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References

Altman, G.H., Diaz, F., Jakuba, C., Calabro, T., Horan, R.L., Chen, J. (2003): Silk-based biomaterials. *Biomaterials*., 24: 401- 416.

Chen, K.P., Huang, J.T., Yao Q. (2014).Model organism *Bombyxmori*. Phoenix Science Press, Nan Jing, China.

Christian, L,, Mounty, John, C., Owens, Brod, E.L., Frank, M.D. (1986). Interaction studies of methyl parathion, carbofuran, permethrin and piperonylbutoxide applied to South Western corn borer Larvae (Lepidoptera, pyralidae). *J. Eco. Entomol*, 79, 531-541.

Finney, D.J. (1971). Probit Analysis. 3rd (ed) London, New York, Cambridge University ress, p: 333.

Forman, S., Kas J., Fini F., Steinberg M., Ruml, T. (1999).The effect of different solvents on the ATP/ADP content and growth properties of HeLa cells. *J Biochem Mol Toxic*ol 13, 11-15.

Hamamoto, H., Urai, M., Ishii, K., Yasukawa, J., Paudel, A., Murai, M., Kaji, T., Kuranaga, T., Hamase, K., Katsu, T., Su, J., Adachi, T., Uchida, R., Tomoda, H., Yamada, M., Souma, M., Kurihara, H., Inoue, M., Sekimizu, K..(2015). Lysocin E is a new antibiotic that targets menaquinone in the bacterial mebrane. *Nat Chem Biol.,* 11,127-133.

Hamamoto, H., Tonoike, A., Narushima, K., Horie, R.,Sekimizu, K. (2009). Silkworm as model animal to evaluate drug candidate toxicity and metabolism. *CompPhysiol C ToxicolPharmacol*., 149, 334-339.

Jan, M., Lien, V., Olivia, E., Buenafe, Peter A.M., de Witte, Camila, V.E., Alexander D.C. (2012) Evaluation of 14 Organic Solvents and Carriers for Screening Applications in Zebrafish Embryos and Larvae. PLoS One., 7, e43850.

Kaito C, Sekimizu K. (2007).A silkworm model of pathogenic bacterial infection. *Drug Des.* . Ther.,1, 89-93.

Kato, T., Kajikawa, M., Maenaka, K., Park, E.Y. (2010).Silkworm expression system as a platform technology in life science. *Appl. Microbiol. Biotechnol*. 85, 459-470.

Kuwana, Z., Sekimizu, N.,Sugiyanna, H. (1967). Effects of insecticides on the silkworm larvae, *Bombyx mori*., L. analysis of the insecticides silkworm larvae relationship by means of subcutaneous injections and topical and oral applications. *Bulletin of Sericulture Experiment Station*, 22, 123-180.

Kashi, K.P., 1972. Tolerance of silkworm (*Bombyx mori*., L.) larvae to organophosphorous in secticides. *J. Sericlture Sci*., 41: 301-304.

Levy N. (2012). The use of animal as models: ethical considerations. *Int. J. Stroke. 7*, 440-442.

Matsumoto, Y. *et al*. (2012) Quantitative evaluation of cryptococcal pathogenesis and antifungal drugs using a silkworm infection model with *Cryptococcus neoformans*. *J Appl Microbiol.,* 112, 138-146.

Mita K. (2008). The genome of a lepidopteran model insect, the silkworm Bombyxmori. *Insect Biochem. Mol. Biol*., 38, 1036-1045.

Nwibo, D.D., Hamamoto, H., Matsumoto, Y., Kaito, C., Sekimizu K. (2015). Current use of silkworm larvae (*Bombyx mori*) as an animal model in pharmaco-medical research. *Drug Discov. Ther*., 9, 133-135.

Renwick, J., Kavanagh, K. (2007). Insects as models for studying the virulence of fungal pathogens of humans,. New insights in medical mycology. Springer, Dordrecht, the Netherlands. pp. 45-67

Suresh, P., Atmika, P., Hiroshi, H., Kazuhisa S. (2017) Advantages of the Silkworm As an Animal Model for Developing Novel Antimicrobial Agents. *Front Microbiol*., 8: 373.

Singh, Y.P., A.S. Srivastava. (1984). Relative toxicity of some insecticides to the larvae of crypto blades gnidiella Miller. *J. Entomol*., 46,346-351.

Srivastava, A.S., Massoli, M.A. (1985). Relative toxicity of some insecticides to the second instar larvae of *Legm antriaab fuscenta* Walker. *Entomon*., 10: 241-244.

Thay, M., P.T. Collins. (1986). Joint effect of temperature and insecticides on mortality and fecundity of Sitophilus pruzae, Cealeopteracuculionidaeo and wheated maize. *J. Econom. Entomol*., 79, 909-914.

Usui, K., Nishida, S., Sugita, T., Ueki, T., Matsumoto, Y., Okumura, H., Sekimizu, K.(2016) Acute oral toxicity test of chemical compounds in silkworms. *Drug Discov Ther*., 10, 57-61.

Venkata Reddy, S., Sivarami R. N., Ramamurthi, R.(1989). Carbonyl effects on the growth and silk quantities of the silkworm *Bombyx mori*., L. *Indian J. Sericulture*, 28, 182-190.

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