COMMENTARY

An important player in brine shrimp lethality bioassay: The solvent

Brine shrimp lethality bioassay is a simple, high throughput cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp (*Artemia salina*).[1] This assay was first proposed by Michael *et al*., and further developed by several groups.[2-4] The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines especially natural plant extracts and etc.[5,6] It's a preliminary toxicity screen for further experiments on mammalian animal models.

One important aspect of this assay is that the solvent used in this assay may give false positive signals due to the toxicity of the solvent itself. It has been well-known that some organic solvent and detergents have high cytotoxcity *in vivo*. A systematic study on how high concentration of solvent affects the results from brine shrimp lethality bioassay and guidance for maximum working concentration of solvents is needed. The article published in this issue "Interference from ordinarily used solvents in the outcomes of *A. salina* Lethality Test" fills this gap very well. In this study, the authors tested the solvent toxicity effect on brine shrimp experimentally. This is a very significant study. The authors concluded that dimethyl sulfoxide (DMSO) is a safer solvent in brine shrimp lethality bioassay and the maximum working concentration of solvents such as DMSO, methanol and Tween 20 was suggested.

One conclusion from this article is that Tween 20 showed the most cytotoxicity in brine shrimp lethality bioassay. Tween 20 is a detergent, which can help to dissolve natural products contains oil. Detergents have long alkyl chains and water-soluble functional groups. Hence they are water-soluble and are able to dissolve hydrophobic compounds with their alkyl chains. They are called surface-acting agents. Commonly used detergents include sodium dodecyl sulfate, Tween 20, CHAPS and etc., But these detergents are in general toxic *in vitro* and *in vivo*. The may denature proteins and inhibit biological processes. So to overcome this problem, it is important to discover new detergents with lower toxicity for brine shrimp lethality bioassay and other bioassays. New low toxicity surface-acting agents were developed in the past several decades. One of them is Nikkol. It has been used in a membrane protein research in which the proteins were dissolved with the help of Nikkol, but still maintains a native-like state. Nikkol is a nonionic detergent and showed low toxicity. This may be a promising new solvent can be used in brine shrimp lethality bioassay.[7]

Another aspect of brine shrimp lethality bioassay is the proper design of experiments. As many other medical researches, organic chemicals are generally used as drug vehicles or carriers in brine shrimp lethality bioassay. However, these compounds often have toxicity or even the pleiotropy *in vitro* or *in vivo*.[8] Hence researches must be aware of their desirable or undesirable effects and proper control experiments should be carefully designed. Otherwise, bias may introduced in bioassays and effect of solvents can be attributed to the tested medicine falsely. In brine shrimp lethality bioassay, both positive and negative groups should be designed. Especially the negative group, which should contain the some percentage of solvent as in the test groups, should be planed carefully. And the effect of the solvent only should be deducted from the test data.

In summary, solvent is a very important player in brine shrimp lethality bioassay. The maximum working concentration of solvents in brine shrimp lethality bioassay contributes significantly to the field. Development of new solvents and detergents and carefully design of experiments will also improve this assay greatly.

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J. Adv. Pharm. Technol. Res.

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Quick Response Code:

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How to cite this article: Wu C. An important player in brine shrimp lethality bioassay: The solvent. J Adv Pharm Technol Res 2014;5:57-8.