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Are cytotoxicity tests in biological studies reliable?

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Abstract :
Cytotoxicity is one of the most common tests used in biological studies to assay whether a compound has some, or any, kind of therapeutic properties on cell types. Although it is a widespread test across disciplines that range from pharmacy to biology and medicine, amongst others, there are factors that alter reliable outcomes. These factors are either laboratory or environmental; and has an effect on the intercellular and extracellular features cell types experience, once they are exposed to different substances. The fluctuations in cytotoxicity outcomes are a result of not only human error, but also the manner in which plants/compounds are prepared during preliminary experiments. However, it's possible for these tests to be conclusive by factoring the phytochemical constituents of plant compounds that have an effect on the genetics of signal transduction events in cells. This implies that the work performed on cytotoxicity is valid, however, several questions pertaining to the reliability of test outcomes still remain. In this paper, the trustworthy nature of cytotoxicity tests in biological studies will be considered.

Keywords: cytotoxicity, incubation, rainfall, stereochemistry, invirtification, intracellular, extracellular, measurements, compounds, reliability.

WHAT IS CYTOTOXICITY?

Cytotoxicity is a scientific term that is used when one measures the toxicity of compounds (or substances) on celled organisms such as bacteria, cancer, viruses or fungi. It is a term that is used to assay compounds for their effect on the mentioned celled organisms, often with a high degree of reliability. However, due to external parameters, sometimes cytotoxicity measurements are doubted by researchers due to confirmatory tests that sometimes refute cytotoxicity results (Singh, 2018). Often in cancer studies, for example, cytotoxicity is said to be a measure of mitochondrial activity. Therefore, although this is true also in other celled types, the reliability of those measurements is questionable because the environments in which they thrive vary greatly compared to them thriving predominately in mammalian hosts (Singh, 2018). Cytotoxicity is thus a tern that is also important to the medical fraternity as it is able to decipher compounds that are harmful and should be discarded in laboratory tests from those which should be further evaluated for their therapeutic, or biological, properties. Although this is an indication of the bioactive properties of a substance, there are many laboratory and environment outliers that affect these tests, and thus, their reliability is often compromised (Singh, 2018).

THE CONDITIONS AFFECTING CYTOTOXICITY TESTS

a) Laboratory conditions

There are many laboratory conditions that affect the outcomes of cytotoxicity tests. One of these conditions is the incubation temperature of the cells studied (Singh, 2018; Rehman *et al*., 2009). Although not many studies have reported that this parameter can affect cytotoxicity test result outcomes, it's possible for compounds to undergo statistical re-configuration in the event of fluctuating temperature conditions (read Oyedeji *et al*., 2009). Although this parameter has been reported in many chemistry studies, in biological studies, it still requires clarification (Singh, 2018). Another condition that could affect the reliability of cytotoxicity outcomes is the ingredients of cell culturing media (Singh, 2018; read Seyydnejad *et al*., 2010; read Bauer *et al*., 1996). A question often answered in cellular studies, such as those in cancer research, is that the chemicals of compounds combined with cell culture media, may or may not dilute the active ingredients of toxic compounds (Singh, 2018). Therefore, a major outlier is that this parameter need be eliminated to obtain accurate growth medium pH conditions (Singh, 2018). Invitrification in micro-propagation should also be avoided to avoid plant compounds becoming contaminated with bacteria or fungi substances (Gopalkrishnan, 2010).

b) Environment conditions

One of the environment conditions that affect the outcome of cytotoxicity tests is the region from where plant material is collected (Sofowora, 1993). This is important to note because sunshine intensity alters the photosynthetic capacity of plants (Singh, 2018). In the case of natural compounds, such as drugs for example, freezing, cooling and storage conditions have an impact on the chirality and stereochemistry of them (Singh, 2018). Thus, cytotoxicity result outcomes in some cases may, perhaps, be the result of enantiomeric compounds. An example of an enantiomeric compound that has been tested for susceptibility is D-cycloserine and L-cycloserine. It has been found that the effect of these two compounds in tuberculosis cells are different (Singh, 2018). Rainfall frequency is also another parameter that affects cytotoxicity outcomes. If rainfall isn't frequent in the location from where plant material is collected, then the compounds found in such mesophytic plants, wouldn't be the same as those found in frequently wet areas. Perhaps the quantity of cytotoxicity compounds between the same plants in different locations would be different (Singh, 2018; Sofowora, 1993; Nazif, 2002; Riazand Chaudhary, 1990) . This implies that cytotoxicity test outcomes in biological studies vary considerably, and that, the two mentioned factors need to be studied in line with cell types chosen to be studied. The third environment condition that affects cytotoxicity result outcomes is the soil conditions. Properly nourished soil would provide good plants, but there is no certainly about the quantity of toxic compounds from them, when compared to less manured soil grown plants of the same species (Singh, 2018).

3. THE STATISTICAL PARAMETERS OF CYTOTOXICITY TESTS

The statistical parameters of cytotoxicity tests are often reliable when working with data sets (Singh, 2018). However, a major factor that must be considered when interpreting statistical results is that plant compounds contain a conglomerate of substances (Sofowora, 1993; Van Zyl *et al*., 2006). In spite of this, the reliability of cytotoxicity outcomes are often reported as being trustworthy when reported, because it's a laborious task to consider biological, biochemical and chemical parameters in all cytotoxicity result interpretations. This means that cytotoxicity results, interpreted using the t-test, non-parametric Analysis of Variance (ANOVA) and the Bonferroni statistic, is confounded by factors well-reported in scientific literature (Singh, 2018). Although these tests are accurate, and thus trustworthy, it's often difficult to trace errors, though human error during cytotoxicity experiments is possible. Some of the human errors that can occur during cytotoxicity tests that affect the statistical parameters that have been mentioned is: pipetting errors when administering compounds to cells (Singh, 2018; Seyydnejal *et al*., 2010; Bauer *et al*., 1996; Van Zyl *et al*., 2006; Chitemerere and Mukanganyama, 2011) (previously mentioned), inconsistent cell counts using the haemocytometer and trypan blue stains (during cancer studies) (Singh, 2018), non-smooth smearing of bacterium

cultures on agar plates (Singh, 2018; read Seyydnejad *et al*., 2010; Bauer *et al*., 1996), the use of old McFarland standards (in TB studies) and the incomplete diluting of plant material used during extract preparation (Singh, 2018) – to name just a few. This means that although cytotoxicity tests in biological studies are trustworthy, they are reproducible, but with a high degree of subjectivity (Singh, 2018). Therefore, compound analysis without the use of statistical results, is reliable from a pharmaceutical point-of-view.

4. THE INTRACELLULAR AND EXTRACELLULAR FEATURES OF CYTOTOXICITY TESTS

The intracellular and extracellular features of cytotoxicity tests are vastly different. Some intercellular features is the collapsing of the cytoskeletal elements, nuclear breakdown and protein malfunction (Singh, 2018). These features are usually exhibited when a compound is toxic to cells of the same kind. This eventually results in the shutdown of certain cellular processes like digestion or vacuolisation by a cell, for example (Singh, 2018). Once the cell dies, as detected by a cytotoxicity test, it also affects tissue production and the function of the systems these tissues form. Due to nuclear breakdown, cells arrest automatically usually due to the process of apoptosis (or unnatural cell death, also known as programmed cell death) (Singh, 2018), however, the reliability of cytotoxicity outcomes here is dependent on whether it was the administered compound that had caused cell death or if it had been some other condition (like the 2 mentioned in section 2) or parameters (Singh, 2018; read Sikkema *et al*., 1994). Some intracellular features of cytotoxicity tests are an interpretation of cytoplasmic streaming, the expulsion of waste products among different cells, the collapsing of cell membranes and the intertwining of them, amongst others. This means that cytotoxic test outcomes would still be reliable in the event of intracellular events occulting following the administration of test compounds, since cytotoxicity is dependent on cell-compound interactions (Singh, 2018; Seyydnejad *et al*., 2010; Bauer *et al*., 1996; read Sikkema *et al*., 1994; Dewhirst, 1980). Thus, intercellular events are of important consideration during the interpretation of cytotoxicity measurements. However intracellular events may obscure those measurements due to additional toxic waste accumulation within cellular cultures. Therefore, the variable to be considered here are interspersed and varied, some of which can't be avoided entirely. This means that in order to obtain a reliable cytotoxicity result, comments on the intracellular events are pivotal, since intercellular events are common features of cytotoxicity tests, even with natural compounds (Singh, 2018).

CONCLUSIONS: TOXICITY, GENETICS AND SIGNAL TRANSDUCTION EVENTS

Although the reliability of cytotoxicity results are confounded by the toxicity of compounds, the genetics of the different cells studied, as well as, signal transduction events, there are factors that hold true to these tests (Singh, 2018). One factor is that a cytotoxicity test is reliable if all events are substantiated in relation to the compound that is being tested (Singh, 2018; Seyyhnejad, 2010; Bauer *et al*., 1996; Gopalkrishnan *et al*., 2010; Riaz and Chaudhary, 1990; Nazif, 2002). This highlights the specificity of certain compounds to different cell types. Furthermore, this highlights that signal transduction events are also compound specific. However, although certain compounds may not be toxic and induce a genetically-related event, like signal transduction events, it may not necessarily confound a cytotoxic measurement (Singh, 2018). However, future tests may show that other factors have influenced cytotoxicity readings. Therefore, cytotoxicity tests in biological studies are trustworthy.

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