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Review Article

Recent Developments in Toxicology Study

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ABSTRACT

The story of toxicology began in the primitive age where our ancestors were fighting the battle of survival, they recognized the poisonous plants and extracted the poison which they used to survive. That was just the beginning and by the 1500BC people already marked opium, hemlock and some certain metals as the dangerous poisonous substances. Toxicology was at first developed as the study of poisons but through the development it is now the study of adverse effect of chemical agents on living organism. The key of any development is to overcome the setbacks and the toxicological development was no exception. Various setbacks appeared through the ages like sulfanilamide catastrophe, irrational use of animals in toxicity study, cross species differences, financial exposures etc. All of these setbacks pushed the human beings to the urge of developing new methodology, technology for the toxicology study and as results various in-vitro study methods, toxicogenomics, toxicoproteomics study emerged.

Keywords: In-vitro study, toxicogenomics, biomarkers, toxicity study.

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INTRODUCTION:

The traditional and descriptive definition of toxicology is "the study of the adverse effects of chemicals or physical agents on living organisms". Adverse effects may occur in many forms, ranging from immediate death to few changes that are not realized until months or years later and it may occur at various levels within the body, such as an organ, a type of cell, or as a form of typical biochemicals. Theknowledge of how toxic substances damage the body has progressed along with medical knowledge. It is now known that various observable changes in body functions actually result from previously unrecognized changes in the form of specific biochemicals in the body.

It is believed, based on available sources that "toxicology" originated from Greek. The Greeks referred to all drugs, potions, and natural products as "pharmaka" or "pharmakon", without distinguishing between those that contained active ingredients and those that didn't.Eventually, the Greek word pharmakon came to mean poison. The Greek term ‘toxicos’ was derived from the noun ‘toxon’, meaning bow. Other Greek terms for toxicology include ‘toxicon’ and toxicos, which mean the poisons dipped into arrows. However, there are some subtle differences in these terms. Like toxicants are any chemical that can injure or kill humans, animals, or plants; a poison. The term “toxicant”is used when talking about toxic substances that are produced by or are a by-product of human-made activities. As example ‘dioxin’ produced as a by-product of chlorinated chemicals. Andon the other way terms like toxin is usually used when the toxic substances produced naturallyAny poisonous substance, whether they are microbial, vegetable, or synthetic chemical, that reacts with specific cellular components to kill cells, alter growth or development, or kill an organism may be considered a toxin.

The history of toxicology presents both diabolical and colourful perspectives on not only the development of medical science but also changed the ancient society’s approach to face in the path of so-called incurable diseases. Toxicology's history began with cave dwellers who recognized poisonous plants and animals, and used their extracts in hunting or for survival warfare. By 1500 BC, written records noted hemlock, opium, and certain metals as dangerous. With time, poisons became widely used and with great sophistication. It was Paracelsus who found that

chemicals in plants or animals caused toxicity. He also proved that the body's response to chemicals depended on the dose. His studies revealed that small doses of a substance were harmless or cause harm depending on how high the dose is. This is now known as the dose-response relationship, a major concept of toxicology. Paracelsus was one of the founders of modern toxicology. Orfila who was a Spanish physician who is referred to as the founder of toxicology. Orfila first prepared a systematic correlation between the chemical and biological properties of poisons of that time. He demonstrated the effects of poisons on specific organs by analysing the autopsy of substances for poisons and their association with the tissue damage.[1]But in the over the past 150 years, poison analysis has made impressive progress. Modern techniques and instruments are now enabling even the tiniest traces of alien compounds to be detected, not just from tissue and organ samples collected at the time of premortem. With time several techniques like isolation techniques, purification techniques, identification techniques, qualitative micro-analysis for elements were used for toxicological analysis.[2]

With the introduction of newer groups of drugs in the market for therapeutic practice, the margin between conventional dose and toxic dose is often becomes narrow and the practitioner must exercise some strict analytical control while administration of drug. The real problems of toxicological study are rarely appreciated except by those intimately connected with the field. The toxic nature of many of the poisonous type of the vegetables and animals are similarly completely uncharacterised.

According to the US National Research Council (NRC), "Toxicology Testing in the 21st Century" proposes a long-term strategy designed to utilize new tools and technologies to allow for the assessment of environmental agents to which human populations may be exposed. Among its key elements were the use of high-throughput in-vitro testing systems and methods in computational toxicology, reducing the proportion of time-consuming and cost-inefficient toxicological studies conducted on animals.[1]

EARLY DEVELOPMENTS:

To know about the development of toxicological study strategies we need to know how they evolved in time and what was the reason behind the evolution. There were some differences that aroused within some federal agencies in time regarding the regulatory toxicology which was one of major reason that lead to the advancement of toxicological studies.

In 1937, the FDA (Food and Drug Administration) acted on a regulation about the labelling law of misbranded drug after the sulphanilamide tragedy that cost 73 lives. In 1962 due to the major amendment of FDCA more vast clinical trials were required to get positive approval from the FDA for the marketing of a drug. Later the FDA was given authority to conduct toxicity studies for food additives to set safety criteria. Due to the need of safety assessments FDA, academe and industry toxicologists developed the first modern protocols in toxicology during the 1950 to 1960. These protocols shaped the toxicity studies that are conducted today. Regarding the regulatory requirements the clinical trials on human is necessary for the approval of drug substances but in case of food additives no such requirement is present. In the late 1970 due to the concern about chemical contamination in the environment the U.S EPA (United States Environment Protection Agency) was founded and the motto was "to protect human health and to safeguard natural environment- air, water and land- upon which life depends". FDA's drug and food additives testing program as well as the EPA's pesticide testing program are strategies designed to promote the safety evaluation of chemicals before use. A notable drawback came under the EPA when the chemical named cyclohexanol was proposed to be tested for toxicity on the 1000 rats. This program was called High Protection Volume Challenge Program (HPV program). A coalition filed notice of intent to sue the EPA against the HPV program charging under the guilty of animal cruelty.

The toxic substances control act defines "an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance." There may be factors to be considered when determining the route of animal exposure, including the route of human exposure, practical difficulties, bioavailability etc. Annually millions of animals are forced upon to get tested for the toxicological studies. In case of LD_{50} study 50% of the test sample is lethal in nature and as a result the exposed animals suffers from acute pain, convulsions, bleeding from eyes and mouth. In the Draize test the corrosive substances are exposed upon rabbits and causes irreparable damage to the skin and eyes. After the decades of toxicity testing of chemical substances on the animals in 1963, the National Institute of Health published a set of guidelines on the care for laboratory animals. And in 1985 an amendment of Animal Welfare Act was passed. The new regulation of guidelines included the use of anaesthesia, medication and proper euthanasia of laboratory animals. The researcher can, however, exclude any of these procedures if it is 'scientifically necessary'.

In the past few decades, the shortcomings of animal testing have arrived. The alternative test methods are applied nowadays to avoid the expense and time and to reduce animal pain and distress. According to some reports, it costs approximately 2,000,000$ for keeping and monitoring of animals for months and years and to involve them into immunotoxicity assay just for a single chemical of one exposure route. Acute toxicity testing approximately costs about 7,000$ for each animal and about 900,000$ for a span of only 2 years. Hence the millions of dollars are required to be invested over a period of year on only one species.

The example of cross-species differences is one of the reasons labelled as the drawback of animal testing. Over 52% of drugs that are marketed over the span of 10 years, resulted in serious toxicity or even fatal side effects that was overlooked by animal tests. The sum is huge. The reason behind this phenomenon is the questionable result in animal testing as the genetic differences among the animals of the same species as well as the humans. The premature approval of the chemicals later led to fatal results. Multicentre Evaluation of In-Vitro Cytotoxicity (MEIC) stated that "while rat and mouse tests were only roughly 65% accurate in predicting human lethal blood concentrations of

chemicals, a combination of human-cell tests predicted chemical toxicity with 80% precision."

In 1997, a regulatory body, The Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) was founded and the functions of the committee were to review and evaluate the proposed alternative test methods and protocols and to provide guidance. But in a practical sense, each of the agencies and committees has different guidelines for many of the same compounds. To avoid such drawbacks in testing program short-term test (STT) have gain popularity. The goal of STT is to identify preliminary lower tier hazards such as chromosomal damage or genetic mutation within weeks and it is also economically efficient. Although this method is less effective in long term but provides strong preliminary screening.[3]

INCLUSION OF IN-VITRO STUDY:

In vitro tests are considered as a revolutionary step for the toxicity study. From the reduced variability of experiments to the high efficiency standardization, many advantages are there in in-vitro tests. In case of testing the degree of toxicity of anticancer drugs cell and tissue cultures are done. It also helps to determine whether the drug is capable enough to cause elimination of cancerous cells. This tissue or cell culture method is easy to handle from microscopic to molecular viewpoint. But there is a condition while performing the study, first we need to study the effect on cells when applied to living organism as in case of in-vivo test many factors like interference of body's own response can be noted. Using in-vitro tests widely reduced the number of animals involved in research. Earlier in case of the treatment of the growth hormone disease in children the extraction of growth hormones from the deceased donors were used. But after finding contamination in some children this method has fell into disuse in 2009. In vitro tissue tests are also carried out by computer models for predicting the metabolic and physiological effects on human body by using large number of equations obtained from the living animal experiments.

Another method that is used widely is the cell variability test. In this method the parameters of viability are studied and the toxicity tests of various substances can be performed. The toxicity test can be performed on chicken embryos, fish and in amphibians and this technique is quite remarkably successful and has proven useful.

The in-vitro data should be considered as quantitative data and information while performing the calculation of initial dose of any drug substances. In the process of determining the oral initial dose the use of Neutral Red Uptake (NRU) approach has proven useful as it reduces the number of animal use while performing the toxicity study.

In case testing of phototoxicity the OECD (Organisation for Economic Co-operation and Development) recommends to use in-vitro study as it reduces the cell variability when the potential phototoxic substances are exposed to light or in the absence of light. The example of in-vitro 3T3 NRU phototoxicity test shows the remarkable results of predicting the acute phototoxicity effects in animals and in human.[4]

There is another method that has been approved by the ICCVAM is the 'Corrositex' method. This method has replaced in some extend the in vivo corrosivity testing of chemicals and it reduces radically the traditional animal use in corrosivity testing. The test is carried out by forming an artificial skin with collagen matrix barrier and the potential toxic substances are tested on that artificial skin. Another similar method named 'EpiDerm' and 'EPISKIN' are carried out for assessing the corrosivity of chemicals in human skin. In this method cell death period of in vitro human tissue culture is recorded when it is exposed to potentially corrosive chemicals. The ability of testing on an artificial human skin without any has introduced a new era of toxicity study.[5,6]

DEVELOPMENT THROUGH TOXICOGENOMICS:

As the development of toxicological study methods are occurred the involvement of toxicogenomics became significantly relevant. In the late 1990's, the evolution in genomics study and understanding the application of gene expression have led to the toxicogenomics. The toxicogenomics has widen the view of predicting the adverse effect at the definite levels of gene expression in the living organism. How genomes respond to chemical and environmental stressors has been characterized by toxic genomics. The profiling of mRNA expression with proteomics has become a way to interpret the role of the gene in environmental interaction in disease. The problems associated with conducting safety and risk evaluations for medications and chemicals as well as identifying environmental stressors that are involved in the etiology of human illness have been challenging concerns for a long time but the genomics technologies have enough potential to alter the traditional way to assess the toxicity risk in human health. Toxicogenomicshas three principles. The first one is to identify the specific biomarkers that can help to expose toxic substances and the second one is to elucidate the mechanism of toxicity at the molecular level and the last one is to understand the relationship between environmental stressors and their capability to cause human disease. The variable data derived from the transcriptomics, proteomics, and traditional toxicological data evaluation integrate into toxicogenomics and it helps to form a relationship between toxicological outcome and molecular genetics.[7]

The toxicogenomics study is beginning to incorporate various studies of data streams like proteomics, transcriptomics and metabonomic. It is rapidly developing from the studies that are done on individual chemicals to knowledge and informatic-based science. There are basically two types of toxicogenomics approaches. The comparative or predictive approach deals with the automated pattern to recognize and analyse the data sets instead of exploring the individual genes for obtaining information. This approach helps in determining the genetic and proteomic variability of assayed samples. The other approach is the functional approach which is the study of the compound effects on genes and proteins of biological organisms. This study is basically done by the mechanistic inference. The mechanistic inference is a sequence of events after the toxicant is exposed on biological cells and it is viewed in dose and time-space. It actually proves that the gene and protein expression pattern is highly dependent on the toxicant concentration. So, the understanding of the mechanism of action of a potential toxicant compound on

the basis of the combination of time and dose can minimize the misinterpretation of transient response and it will allow the identification of delayed alterations which might represent the suspected biomarkers of pathophysiological endpoints.[8,9]

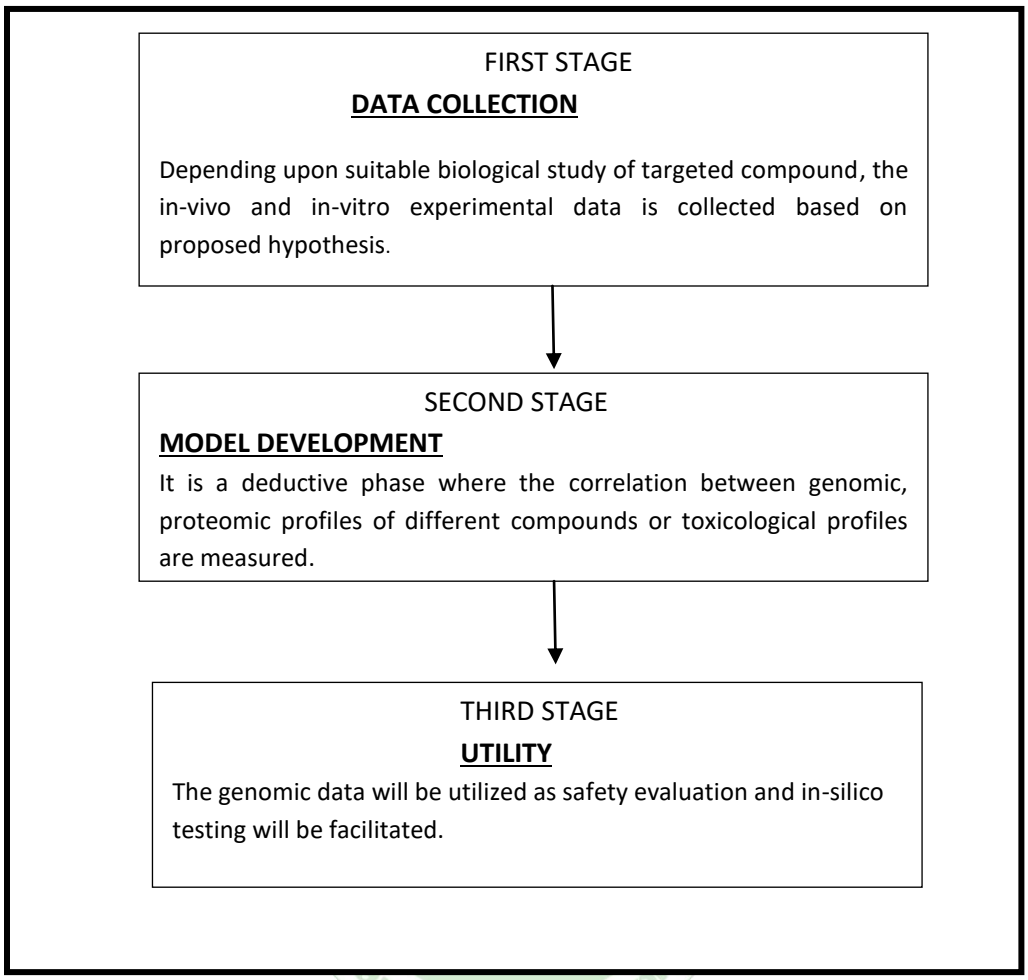

Figure 1: Stages of Comparative/ Predictive Model

Another aspect of the toxicogenomic study is to implementation of biomarkers that can indicate disease, adverse response, and pharmacological response to some certain stressors or toxicants. To predict liver toxicity some number of toxicity relevant gene that are found potential to be biomarkers. Serum ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), Alkaline phosphate, glutamyl transpeptidase, and ornithine carbamoyl transferase are examples of such biomarkers. In cancer diagnosis, the implementation of next-generation sequencing or NGS has gained significant traction. Although a great number of toxicity relevant gene expressions have been found through the reports of toxicogenomics study of various animal models and that can be established as potential toxicogenomics biomarkers for hepatotoxicity. As for an example acetaminophen and carbon tetrachloride have been widely used as a potential toxicant in toxicogenomics study for hepatotoxicity and a set of genes that can be associated with liver cell injury have been reported. The practical approach for the application of toxicogenomics biomarkers is to identify and prioritize the suspected drug candidates according to the microarray data.[10,11]

CONCLUSION:

A defining point event that builds on the past and future in a new era is a common component of development. From the discovery of penicillin to the clarification of the DNA double helix, all had unlucky beginnings and then over the course of years, overcoming the drawbacks now they are enlisted as a lifesaving drug or a map of human genomic sequence. The advancement of toxicology is no exception. Step by step the toxicology study has become a pivotal stage of any drug development process. Prior to being used on humans, newly developed medications must undergo extensive toxicity testing. The goal of toxicity testing is to identify any potential hazardous consequences that a test chemical may have, not merely to determine how safe it is and to determine how test compounds affect lab animals and whether they have any direct hazardous effects on humans. The inclusion of subjecting lab animals to high dosages in order to assess any potential risks to humans who are introduced to much lower dose. although with the help of constant development in new alternate methods are available in the field of toxicology. Implementation of toxicogenomics methods in toxicity testing can

revolutionize the toxicology study. RNA profiling of formalin fixed tissues is already being used for gene expression analysis. Use of gas chromatography, liquid chromatography or mass spectrometry in array of several hundreds of toxicologically active protein antibodies is being done. Evaluation and identification of proper biomarkers which is more sensitive and more accurate can be done by the toxicoproteomics research. Individual genotype, lifestyle, age, and exposure history are taken into account when determining the toxicogenomic response to environmental exposures. The extent to which these factors can affect the balance between healthy and non-healthy state can be assessed by the toxicogenomics study. Through the global monitoring of genetic reactions with therapeutically and environmentally relevant dose-regimens, toxicogenomics will strengthen the relevance of toxicology.

Declaration of conflicts of interest - The author has no conflicts of interest to declare. I agree with the contents of the manuscript and there is no financial interest to report. I certify that the submission is original work and is not under review at any other publication.

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