A Review on Genotoxicity of Different Drug Molecule

By

S.M. Mahmud Jahan Rumi
14346008

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy (Hons.)

School of Pharmacy

BRAC University
November, 2022

Declaration

It is hereby declared that

1. The work which is being presented in the thesis entitled **"A Review on Genotoxicity of Different Drug Molecules"**, in partial fulfillment of the requirement for the award of the degree of Bachelor of Pharmacy and submitted to BRAC University.

2. The thesis submitted is my own original piece of research work under the guidance Dr. Md. Aminul Haque, Associate Professor, School of Pharmacy, BRAC University.

3. The thesis does not include any already published or written by third party content, unless it is properly cited in the references.

4. The matter embodied in this thesis has not been accepted or submitted by me for any other degree or diploma at a university or other institution.

5. I have acknowledged all my primary sources of assistance.

S.M. Mahmud Jahan Rumi

14346008

Approval

This undergraduate thesis entitled "Genotoxicity of Different Drug Molecule" prepared and submitted by S.M. Mahmud Jahan Rumi (ID- 14346008) of summer, 2022 in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy has been examined and is recommended for acceptance and approval for oral defense.

Supervised By:

Dr. Md. Aminul Haque
Associate Professor
School of Pharmacy
BRAC University

Approved By:

Program Director:

Professor Dr. Hasina Yasmin
Program Director and Assistant Dean
School of Pharmacy
BRAC University

Dean:

Professor Dr. Eva Rahman Kabir
Dean
School of Pharmacy
BRAC University

Ethics Statement

The author stated that there were no professional or other personal interests of any nature or kind in any product, service or company that could be interpreted as influencing the position presented in or the review of the manuscript titled, "Genotoxicity of Different Drug Molecule." Author also certified that I have no financial or personal ties to individuals or organizations that could improperly influence this work.

Abstract

In the field of genetics, genotoxicity is a property of chemical compounds that results in mutations by harming a cell's genetic material. In genetics, it's also used to define a harmful reaction on a cell's genetic substance (DNA, RNA) that changes the integrity of the cell. Mutagens are substances that are responsible for mutations. Radiation and chemical genotoxins are both examples of genotoxins. A genotoxin is a substance that possesses the property of genotoxicity. Genotoxic impurities (GTIs) are chemicals that can cause cancer by causing genetic mutations, chromosomal breakage, and/or chromosomal rearrangements. The main issue is that genotoxic substance of drug may trigger somatic mutations or cause cancer. Drug substances are vulnerable to chemical reactions with other components such as excipients or their contaminants, drug substance counter ions, residual solvents, and degradation reaction side-products. This review study focuses on the necessity for genotoxicity, the importance of genotoxicity, genotoxicity mechanisms, genotoxicity testing and genotoxicity evaluation. Besides, this paper also reviews the genotoxicity of some different drug molecule.

Key words: Genotoxicity, Mutation, Toxicity, Carcinogenicity, Enzymes, Impurities, Molecule, Substance.

Acknowledgements

I am grateful to almighty Allah for providing me the opportunity to work with such wonderful people from the school of pharmacy who have always been idealistic and encouraging throughout my journey.

First and foremost, I am indebted to my supervisor Dr. Md. Aminul Haque (Associate Professor, School of Pharmacy, Brac University) for giving me the privilege to work as one of his thesis students. In addition, his support, guidance, dedication, enthusiasm and expertise in this arena have driven me more interested in thesis work and helped me to complete the research properly.

Secondly, I would like to thank Professor Dr. Hasina Yasmin (Program Director and Assistant Dean, School of Pharmacy, BRAC University) for providing me with huge knowledge to make my journey easy and convenient. Most significantly, I would love to thank Professor Dr. Eva Rahman Kabir (Dean, School of Pharmacy, BRAC University) for her support, motivation and kind words. Furthermore, I am grateful to all the faculty members of School of Pharmacy, BRAC University for their enormous efforts for the accomplishment of my graduation. Finally, I want to express my eternal gratitude to my friends and seniors for their guidance and my family members for supporting me all the way.

Table of Contents

List of Tables

List of Figures

List of Acronyms

DNA	Deoxyribonucleic Acid
CA	Chromosomal Aberration
NCE	New Chemical Entities
NDA	New Drug Application
NPs	Nano Particles
NOCs	N-Nitroso compounds
ADME	Absorption Distribution Metabolism Excretion
FA	Formaldehyde
SCE	Sister Chromatid Exchanges
DPX	DNA- Protein Crosslinks
MNT	Micronucleus Test
LSD	Lysergic acid Diethylamide
MN	Micronucleus
WHO	World Health Organization
SCE	Sister Chromatid Exchange
CSC	Cigarette Smoke Condensate

HPRT	Hypoxanthine Guaninephosphoribodyl Transferase
AEs	Adverse Events
APAP	N-acetyl-para-aminophenol
DC	Diclofenac

Introduction

In the field of genetics, genotoxicity is a property of chemical compounds that results in mutations by harming a cell's genetic material. In genetics, it's also used to define a harmful reaction on a cell's genetic substance (DNA, RNA) that changes the integrity of the cell. Mutagens are substances that are responsible for mutations. Radiation and chemical genotoxins are both examples of genotoxins. A genotoxin is a substance that possesses the property of genotoxicity. Genotoxic impurities (GTIs) are chemicals that can cause cancer by causing genetic mutations, chromosomal breakage, and/or chromosomal rearrangements. There is proof that genotoxic chemicals can attach directly to DNA as well as act indirectly by inhibiting DNA replication enzymes. The main issue is that genotoxic substance of drug may trigger somatic mutations or cause cancer. Drug substances are vulnerable to chemical reactions with other components such as excipients or their contaminants, drug substance counter ions, residual solvents, and degradation reaction side-products.

Regulatory bodies all around the world require medications to be tested for gentoxicity prior to marketing. Recent publications in Germany have compiled the results of genotoxicity trial performed on novel chemicals and pharmaceutical submission. These researches show how the various assessments fared both individually and collectively in identifying genotoxic concerns (Snyder & Green, 2001). Typically, a three or four-test battery is required, consisting of an in vivo chromosome stability experiment, chromosomal aberration analysis, in vitro mammalian mutagenesis, bacterial mutagenesis and in vitro chromosomal aberration analysis. In order to make informed risk decisions from the perspective of medication development, it is essential to fully comprehend the mechanism underlying any promising results in genetic toxicity.

The evaluation of genotoxicity is a vital aspect of non-clinical safety evaluation during the development of new drugs. There is much advice on the appropriate testing methods for determining genotoxicity for conventional small molecule therapies (Thybaud et al., 2016).

Despite the fact, epidemiological studies with the exception of anticancer cytotoxics, support the notion that genotoxicity and rodent carcinogenicity are connected with a higher risk of human carcinogenicity. However, it is imperative to proceed cautiously with the discovery of drugs with favorable genotoxicity profiles for the time being.

Need of Genotoxicity

Traditionally, genotoxicity testing have been used to predict carcinogenicity and heritable mutation. Considering that sponsors of botanical drugs should be inspired to gather this data early in the improvement of their products because genotoxicity studies are highly reproducible, comparable in cost (to animal toxicity studies) and have a high statistical power (We et al., 2010). Drug approval and registration necessitate a thorough examination of their genotoxic potential. In many countries, genotoxicity studies are the practice of regulatory toxicity evaluation. Genotoxicity experiments are carried out on bacteria and somatic cells to determine the action of mutations or chromosomal abnormalities occurring as a result of the usage of proposed medications. In recent times, genotoxicity testing has progressed to earlier stages of drug development in order to find genotoxic hazards as quickly as possible.

Importance of Genetoxicity Testing

Genotoxicity and mutagenicity tests are crucial in determining the potential risks of therapeutic medications, cosmetics, agrochemicals, factory substance, food items, common poisons and nanomaterials for regulatory goals. Genotoxicity studies is important prior to phase I and phase II clinical trials as part of the preclinical safety assessment of new medications. Different in

vitro and in vivo approaches use a variety of genotoxicological endpoints including changes in chromosomal number and shape as well as point mutations to assess mutagenicity or genotoxicity (Turkez, H., Arslan, M.E., and Ozdemir, O., 2017). It's made to spot genetic harm like gene mutations and chromosomal detachments, which could indicate the drug's tumorigenic or heritable mutation potential. Pre-clinical research is typically done to determine the fundamental toxicological profile of novel chemical entities (NCE). The safety and effectiveness of NCE are assessed using toxicological data, which will aid in anticipating the drug's potential risk/benefit analysis throughout the New Drug Application (NDA) process (Savale, 2018). These experiments have mostly been used to identify genotoxicity and carcinogenicity.

Genotoxicity Mechanism

Engineered nanoparticles (NPs) are frequently used in a variety of technologies, yet their peculiar features may have negative health consequences. We address probable pathways of genotoxicity generated by NPs by examining recent in vivo and in vitro genotoxicity research. The main medications for genotoxicity include DNA reconstruction mechanisms as well as the metabolism of dangerous chemical clastogens and the use of anticancer medications. For instance, high valent chromium is considered to be a carcinogen because studies have shown that in vivo generation of DNA damage leading to cancer in chromate exposed human populations is related to the mechanism of damage and base oxidation products for the interaction between high valent chromium and DNA. Alkylating compounds, intercalating agents and enzyme inhibitors are medications used to treat genotoxicity and function as anti-cancer agents (Kawanishi. M., Matsuda, T., & Yagi, T., 2014).

Genotoxicity Testing

The main goal of genotoxicity analysis is to see if a substance has the potential to harm genetic material or cause cancer. All regulatory agencies suggested minimal batteries of genetic toxicology tests comprise at least two or three test techniques for identifying genotoxic carcinogens. The genotoxicity of numerous chemical compounds has been assessed using a number of in vivo and vitro systems. Primary genotoxicity can be identified by in vitro investigations, whereas secondary genotoxicity can be identified through an in vivo method, mostly through oxidative stress and inflammation (Turkez et al., 2017). Besides, the evaluation of genotoxicity is a critical component of all sorts of chemical safety assessments.

1. Ames Test

In laboratories, bacterial reverse mutation testing which is also known as the Ames assays is used to identify gene mutation. To compare the various changes in the genetic material, the

approach employs a variety of bacterial strains. Experiment can be executed relatively quickly and easily by planting out His- (negative) mutants on an agar plate that contains with just a trace of histidine (Bencko, 2004). The majority of genotoxic carcinogens and genetic mutations are found in the tesy's result, which include base substitutions and frame shifts as kinds of mutation.

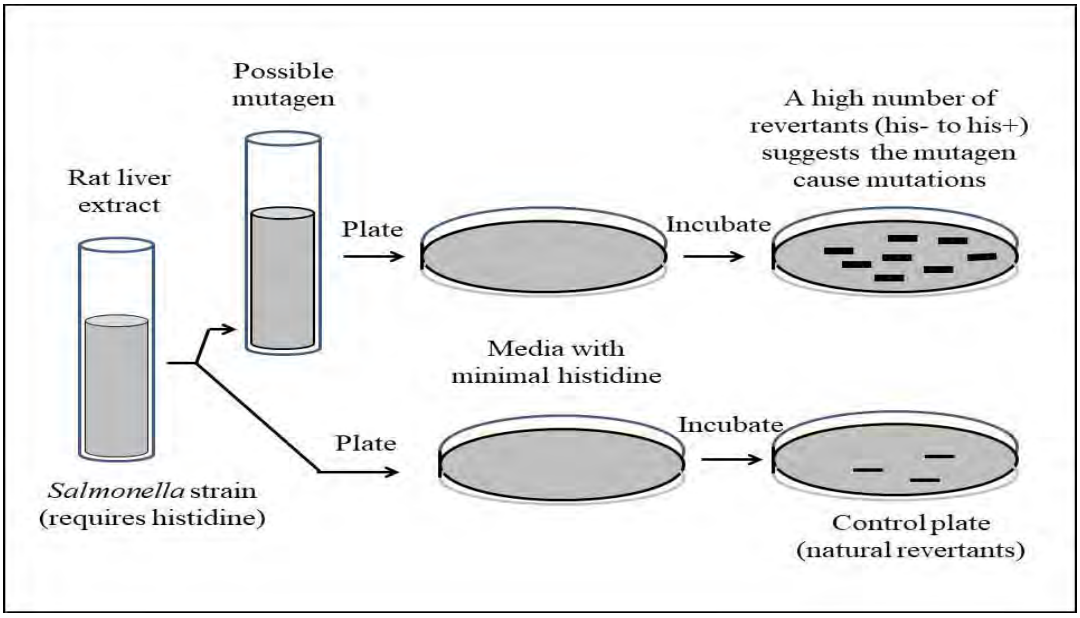

Figure 1: Ames assay method to examine the presence of gene mutation in different bacterial strains *(Savale, 2018)*

2. Chromosomal Aberration Testing

The chromosomal aberration (CA) test is used to determine whether a test chemical has the ability to cause structural chromosomal abnormalities including breaks and exchanges. It is performed in-vitro cultured mammalian cells. Microscopic analysis of chromosomes in mitotic metaphase cells scores structural and numerical damage (Radhika & Jyothi, 2019). Either primary human peripheral blood lymphocytes (HPBL) or established cell lines like Chinese Hamster Ovary (CHO) cells can be used for the chromosomal aberration test.

3. In Vitro Toxicology testing

In vitro testing is used to see if a substrate, a product, or an environmental component causes genetic harm. Early in the development process, assays based on in vitro toxicogenomics can identify a possible genotoxicant's specific mode of action. Three in vitro tests are one for

including gene mutations in bacteria, one for including gene mutations in mammalian cells and one for chromosomal abnormality or micronucleus have been recommended as the minimum

standard for testing across many regulatory sectors (Corvi & Madia, 2016). The purpose of such a battery is to identify gene mutations, as well as structural and numerical chromosomal damage.

Moreover, new in vitro procedures are being created, which provide greater precision. Although they can already be used to assess the relevance of the information obtained with the standard assays (e.g., differentiating DNA reactive from DNA non-reactive compounds) and to better understand modes of action, they have not yet been able to completely replace the regulatory tests currently used in all fields (Corvi & Madia, 2016). Animal experiment for genotoxicity judgment will most likely be reduced and possibly replaced in the short to medium term as a result of a pragmatic procedure that uses sound scientific justification to develop the modern assay paradigm in a way that acceptable to both the regulatory and regulated society.

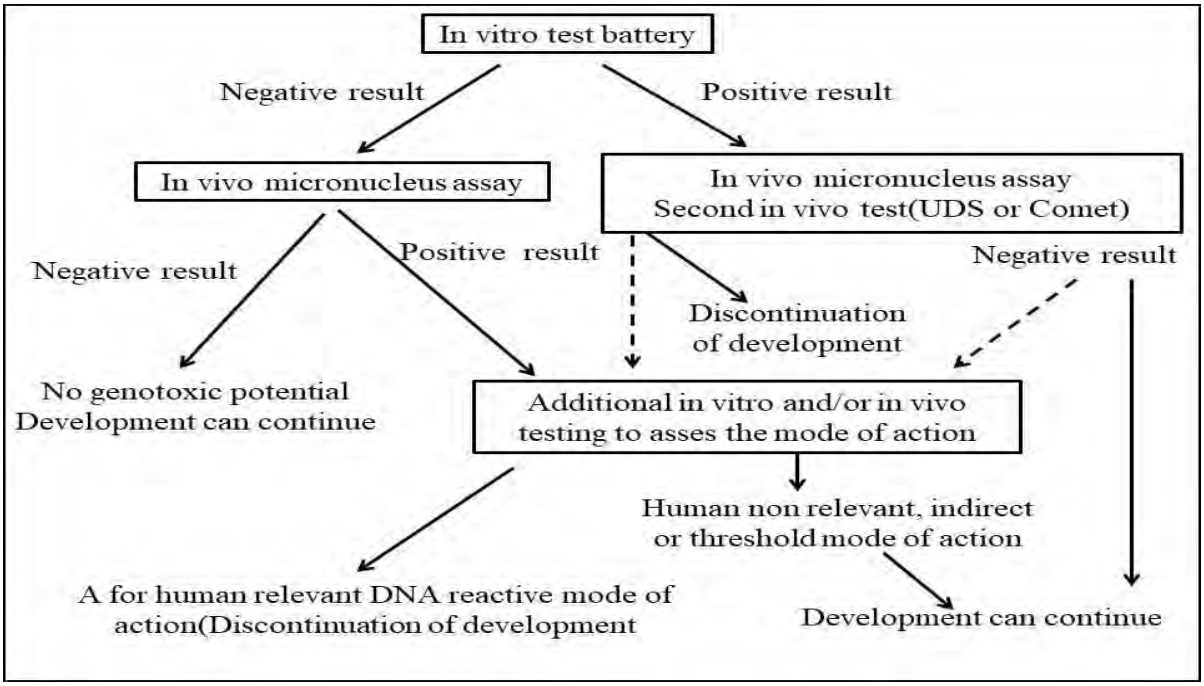

Figure 2: A schematic overview of the decision tree and any necessary follow up testing for regulatory genotoxicity in case of drugs intended for human use (Radhika & Jyothi, 2019).

4. In-vivo Toxicology Testing

The goal of in vivo analysis is to identify the possibility of DNA destruction that could impact chromosomal formation or disrupt the mitotic machinery that turns changes chromosomal number changes. Chromosome number changes resulting ADME and DNA repair are two mechanisms that could influence genotoxicity. It can also discover genotoxic substances that in vitro testing have missed. A micronucleus is a microscopic organelle that exists independently of the nucleus and is composed of nuclear DNA produced from incomplete or lost chromosomes or DNA fragments. The loss of chromosomes during mitosis (aneugenicity), mechanical issues resulting from chromosomal deterioration and change, the loss of acentric chromosomal fragments during mitosis (clastogenicity), and apoptosis are the causes of this structure (Savale, 2018). The in vivo micronucleus experiment checks for chromosomal abnormalities in mammalian cells both for structural and numerical same like the in vitro experiment.

Assay	Sensitivity* (%)	Specificity* (%)	Predictivity* (%)
Ames	58.8	73.9	62.5
Chromosome Aberration (CA)	65.6	44.9	59.8
In-Vitro	78.7	30.8	67.8
In-Vivo	40.0	75.0	48.0

Table 1: The assays of the standard regulatory test battery for the experiment of genotoxicity potential's sensitivity, specificity and predictivity (Radhika & Jyothi, 2019).

* Sensitivity (Percentage of carcinogens positive found in the test)

Specificity (Percentage of carcinogens negative found in the test)

Predicitivity (Percentage of all compounds tested that were accurately predicted)

5. Comet Assay

Alkaline single cell gel electrophoresis is a very helpful tool for diagnosing nanoparticle genotoxicity in drug delivery systems and is also highly recommended to reduce side effects of these types of therapies. This is because there have been many studies on the genotoxic effect of nanomaterial and the high potential of the comet assay in detecting precise DNA destruction.

A sensitive technique is the comet assay, commonly known as single cell gel electrophoresis (SCGE) which is for identifying strand defects in a cell's DNA. It has used in genptoxicity testing. Molecular epidemiology, and basic DNA damage and repair research (Vandghanooni & Eskandani, 2011). The comet assay has become a standard experiment in the battery of assays used to evaluate the safety of new medications and other substances. White blood cells and tissues that can be divided into single cell suspensions provide the raw materials for in vivo research (Collins, 2004).

Genotoxicity of Some Drug Molecule

1. Formaldehyde

Formaldehyde (FA) is one of the most commonly encountered environmental mutagens in humans and it has been found to cause cancer in laboratory animals. It reacts as an electrophile

with guanine and adenine in DNA, resulting in a variety of DNA lesions (Kawanishi et al., 2014). The genotoxicity of FA has been testing using many different ways.

Numerous in vitro investigations have proven that FA is genotoxic. In proliferating cultured mammalian cells, FA caused a variety of genotoxic consequences. The predominant DNA modifications after FA exposure, according to a variety of studies, are DNA–protein crosslinks (DPX). In growing cells, DPX can stop DNA replication and cause additional genotoxic consequences such as sister chromatid exchanges (SCE) (Merk & Speit, 1998). Mutations can develop as a result of incomplete DPX repair (Speit & Schmid, 2006).

Compared to other environmental mutagens, formaldehyde's acute cytotoxic effect is strong and its mutagenicity is relatively weak in previous studies using bacteria cultured cells and animals. However, FA exposure levels would not have been high enough to allow for the patenting of the mutation spectrum's characteristics. Using shuttle vector plasmids, FA induced mutations in human cells were examined (Kawanishi et al., 2014). The micronucleus test (MNT) with exfoliated epithelial cells has been used in numerous investigations to bio monitor genotoxic effects from occupational and environmental exposures. The MNT with exfoliated cells is intended to be a site specific indicator of genotoxic agent exposure and cancer risk, as well as a valuable tool for establishing human exposure limits for genotoxic compounds (Speit & Schmid, 2006).

2. N-Nitroso Compounds

N-Nitroso compounds (NOCs) are a large family of chemical mutagens, carcinogens, teratogens and immunotoxic agent that have been identified as a substantial health risk. Drug which contains n-nitroso groups in drugs, such as secondary, tertiary amines and amides, can create NOCs when they interact with nitrite (Ozhan & Alpertunga, 2003).

3. Alkylating Agents

Alkylated DNA does not coil or uncoil properly, and information-decoding enzymes cannot process it. This causes cytotoxicity, which inhibits cell growth and initiates programmed cell death, also known as apoptosis. However, mutations are triggered as well, including carcinogenic mutations, which explain why which explain why cancer is more common after exposure. Anticancer medicines, for example, Propiolactone, Dimethy sulfate, Diepoxybutane cyclophosphamide, chlorambucil, bleomycin, nitrosources and others (Bencko, 2004).

4. Gastrointestinal Drugs

Numerous patients take medications for gastrointestinal diseases continuously or occasionally for extended periods of time. When comparing the advantages of these treatments, it is important to take into mind the potential for genotoxic and/or carcinogenic side effects.

Five GI AEs (nausea, vomiting, constipation, diarrhea and abdominal pain) rank among the 12 most common when incidence of drug induced adverse events (AEs) is split down by specific symptoms (Peters et al, 2020). It is difficult to measure gastrointestinal toxicity, especially in the colon, small intestine, and stomach. Creative Biolabs has the capacity to offer a comprehensive service of both in vivo and in vitro test methodologies.

A remarkably large number of medications have the potential to directly or indirectly harm one or more gastrointestinal tract segments, leading to one or more clinicopathologic entities. Despite the fact that some medications result in pathology that can be recognized in biopsies or surgical excision specimens, many more medications cause nonspecific pathology. The medications most frequently linked to gastrointestinal harm are NSAIDs. The extensive use of NASAIDs is primarily to blame for this relationship. NSAIDs are among the most frequently

prescribed medications in the globe; in the US, 5-10% of adults (15-25) million people) regularly use an NSAID (Pusztaszeri, Genta, Cryer, 2007).

The stomach should be especially susceptible to harm from drugs because it is a slow transit organ where ingested compounds might stay for several minutes to several hours (Pusztaszri et al., 2007). Due to its often mild and ambiguous symptoms, the frequency of drug induced injury to the small intestine has long been underreported. A remarkably large number of medications have the potential to directly or indirectly harm one or more gastrointestinal tract segments, leading to one or more clinicopathologic entities. A proper index of suspicion in these circumstances, together with effective clinician pathologist communication and a through patient history are necessary for a proper diagnosis (Pusztaszeri et al., 2007).

5. Acetaminophen

The principal uses of acetaminophen, commonly referred to as paracetamol and N-acetyl-p-aminophenol (APAP), are the management of pain and/or fever. Although widely used over the counter analgesic paracetamol is thought to be extraordinarily safe at therapeutic levels. It is also known that at greater doses it can induce acute liver necrosis in experimental animals and humans (Dybing et al., 1984).

Gene mutations are not brought on by paracetamol in either human or bacterial cells. However, there is published data that shows paracetamol damages chromosomes in mammalian cells in vitro at high concentrations and that these effects are also seen in vivo at large dosages (Bergman, Muller, Teigen., 1996). The reactive metabolite of paracetamol has been shown in vitro and in vivo studies to bind permanently to DNA and result in DNA strand breaks. In vitro and in test animals, paracetamol impairs the production of both replicative DNA and DNA repair (Rannug, Holme, Hongslo, Srim, 1995). Paracetamol should be further investigated for

potentional mutagenic effects according to the current findings and a report that it may cause chromosome abnormalities in hamster cells in vitro (Dybing et al., 1984).

6. Diclofenac

Diclofenac (DC) is a non-steroidal anti-inflammatory medicine that was first discovered many years ago and is now widely used. Approximately one billion patients have used it, and it has shown to have an acceptable safety profile.

Based on the data collected throughout the investigation, it is reasonable to draw the conclusion that DC operates as a clastogen in vivo and creates chromosomal fragments that lead to micronuclei and germ cell damage at high dosages and for long periods of time (Rina, Pankaj & SS, 2014). To support the marketing clearance of DC, a broad range of standard and supplemental in vitro and in vivo investigations have been carried out (Hartmann, Erkman, Maremanda, Elhajouji &Martus, 2021).

Genotoxicity of Abused Drugs

Although several studies on misused drugs have been undertaken, the majority of them focus on the issues of addiction and neurotoxicities. Physical and/or psychological dependence is a characteristic of drugs or compounds with abuse potential. Individuals who abuse these medications or frequently relapse due to positive (euphoric) and negative (withdrawal) reinforcement (Li & Lin, 1998). In this overview, the possible genotoxicity of commonly abused substances such as LSD, morphine, cocaine, cannabis is discussed. Besides, the genotoxicity of tobacco and alcohol will also be explored.

1. Lysergic acid diethylamide (LSD)

Acid also known as lysergic acid diethylamide (LSD) is a hallucinogen. LSD frequently causes intensified thoughts, feelings and sensory perception. When used in sufficiently large dosages, it primarily results in visual and hearing hallucinations.

LSD propensity to generate chromosome abnormalities in vitro (cultured human leukocytes) and in vivo (leukocytes from LSD users) initiated different research into the drug's cytogenetic effects (Li & Lin, 1998). Translocations, deficits, duplications, and inversions are all known side effects of chromosome breaking, and if they damage the germ line, they can lead to abnormal meiotic activity. This may boost the number of fetal deaths or births of malformed children (Yujra et al, 2015). In humans, removal of sections of the chromosome can impact the phenotypic. For instance deletion of the short arm of the number 5 chromosome can produce cridu- chat syndrome, and deletion of the long or short arm of the number 18 chromosome can result in congenital abnormalities and mental retardation (Yujra et al, 2015).

2. Morphine

Morphine is a parent parent chemical and metabolite of diacetylmorphine (heroin) that is commonly used to treat moderate to serve pain. Despite the lack of data, in vivo treatment of morphine to mice has been shown to enhance the incidence of chromosome abnormalities in bone marrow cells and cause micronuclei in bone marrow cells and lymphocytes. On the other hand, in vitro morphine therapy, on the other hand, did not result in chromosome abnormalities in cultured human lymphocytes or micronuclei in mitogen stimulated mouse splenocytes (Yujra et al, 2015).

3. Cocaine

Cocaine is a significant alkaloid extracted from coca leaves that is commonly abused in Western countries. In developing rat brain areas, cocaine has been reported to impede DNA synthesis. A single dose of cocaine (60 mg/kg) caused liver injury in male albino Swiss mice and which was described by particular abnormalities in DNA pody and apoptosis (Li & Lin, 1998). Besides, the comet and micronucleus (MN) assays were used to assess the genotoxicity and mutagenicity of crack cocaine, respectively, as a reliable method for detecting genomic damage that was less time less time consuming than other traditional genetic study techniques (Yujra et al, 2015).

4. Tobacco

The leaves of the tobacco plant are fermented and dried before being utilized to produce tobacco products. Nicotine, a component of tobacco that and cause addition, explains why so many people struggle to quit smoking. According to World health Organization (WHO) claims that smoking is one of the worst public health risks in the world, killing more than 8 million people a year of which 1.2 million are women through secondhand smoke

Smokers have considerably show more sister chromatid exchange (SCE), chromosomal abnormalities, and micronuclei in cultured lymphocytes than non-smokers (Pursiainen, 2004). Numerous studies have shown that cigarette smoke condensate (CSC) and cigarette smoke cause rodent DNA strand breakage and mammalian cell culture anomalies and DNA in vitro (DeMarini, 2004). DNA adducts urine metabolites of carcinogens, urinary mutagenicity, SCE, and hypoxanthine guaninephosphoribodyl transferase (HPRT) gene alterations (in neonates exposed through the mother's involuntary smoking) have been identified in humans with involuntary tobacco smoke exposure (Pursiainen, 2004). SCE has

been found in the bone marrow of animals that have been exposed to cigarette smoke. However, there have been some negative researches and one positive research on the production of chromosomal abnormalities in lung cells as a result of such exposure. Nonetheless, micronuclei have been seen in bone marrow, peripheral blood erythrocytes and the lungs as a result of this exposure (DeMarini, 2004).

5. Alcohol

Despite the fact that alcohol is designated as a human carcinogen. Any of the alcohols can cause respiratory depression, aspiration, hypotension, and cardiovascular collapse when consumed in large amounts.

Oxidative metabolism breaks down ethanol virtually entirely in living things (Thybaud et al., 2016). The ethanol micronuclei (MN) assessment is a genotoxicity test that simultaneously provides data on a number of chromosomal damage endpoints such as chromosome breakage, nucleoplasmic bridges (NPBs) and nuclear based gene amplification (Thybaud et al., 2016). Acetaldehyde, the initial result of ethanol metabolism is thought to be the cause of alcohol-induced DNA damage. The ability of acetaldehyde to introduce more intricate DNA alterations such DNA-DNA cross links and DNA protein adducts is a key feature of genotoxicity (Kotova et al., 2012).

Prevention of Genotoxicity

Some chemical compounds can cause fragile spots in oncogene-rich areas of the chromosome which potentially leading to carcinogenic effects. Cancer can develop if genotoxic events like deletions, breaks and rearrangements do not promptly cause cell death. Fragile spots, which are vulnerable to breakage, can be caused by genotoxic chemicals (such as pesticides) (Radhika & Jyothi, 2019).

Reactive oxygen species are produced during the metabolism of some substances, and this process may operate as a genotoxic mechanism. Arsenic's metabolism demonstrates this by generating hydroxyl radicals, which have been connected to genotoxicity. Several medications including busulfsn, carmustine, etoposide etc are being employed as anti-mutagenic actions. Pro-oxidant chemicals have demonstrated the ability to inhibit the manifestation of genotoxic activities by plant-derived polyphenolics and other antioxidant chemicals (Radhika & Jyothi, 2019). Simple phenolics (C6), phenolic acids (C6-C1), cinnamic acid and related compounds (C6-C3) which are non-flavonoid chemicals also have antimutagenic properties (Bhide, Shiatapurkar, Gothoskar, Ranadive, 1989).

Conclusion

In general, genotoxicity testing of drug molecule is an essential part of the security evaluation of wide range of substances including pharmaceuticals, industrial chemicals, pesticides, biocides, food additives, cosmetics products, and veterinary drugs and is important in the situation of global legislation aimed at protecting human and animal health. Recent technological and molecular science advancements have made it possible to investigate complicated chain of biological path at the genome level in return to chemical therapy.

Furthermore, genotoxicity has the possibility of providing judgements on molecular activity of many kinds of toxicants by studying gene explanation profiles. Genotoxic substances alter chromosomal structure by adding, deleting, duplicating, generating rings, and so on. The mutations have the potential to lead to variety of disorders, including cancer. Because of the utilization of improved technology, drug research and development is now more quick, time-saving, and productive. To avoid the possible harm that genotoxicity can bring, it is needed to conduct genotoxicity studies. Moreover, the identification of genotoxic chemicals aids our understandings of the mechanism of mutation and genotoxicity. In this study, we look at how developing toxicogenomic science can be used to assess genotoxicity and carcinogenicity processes in vitro and in vivo test methods. We also talk about potential future applications and viewpoints for these approaches in risk identification and risk assessment paradigms.

Reference

Snyder, R. D., & Green, J. W. (2001). A review of the genotoxicity of marketed pharmaceuticals. *Mutation Research,* 488, 151-159.

Thybaud, V., Kasper, P., Sobol, Z., Elhajouji, A., Fellows, M., Guerard, M., Lynch, A. M., Sutter, A., & Tanir, J. Y. (2016). Genotoxicity assessment of peptide/protein-related biotherapeutics: Points to consider before testing. *Mutagenesis,* 31, 375–384.

Wu, K. M., Dou, J., Ghantous, H., Chen, S., Bigger, A., & Birnkrant, D. (2010). Current regulatory perspectives on genotoxicity testing for botanical drug product development in the U.S.A. *Regulatory Toxicology and Pharmacology,* 56, 1–3.

Turkez, H., Arslan, M. E., & Ozdemir, O. (2017). Genotoxicity testing: progress and prospects for the next decade. *Expert Opinion on Drug Metabolism and Toxicology,* 13, 1089–1098.

Savale, S. K. (2018). Genotoxicity of Drugs Introduction Predictionand Evaluation. *Asian Journal of Biomaterial Research,* 4, 1-9.

Kawanishi, M., Matsuda, T., & Yagi, T. (2014). Genotoxicity of formaldehyde: Molecular basis of DNA damage and mutation. In *Frontiers in Environmental Science*, 2, 36.

Bencko, V. (2004). *Hygiene & epidemiology : selected chapters*. Karolinum.

Radhika, P., & Jyothi, Y. (2019). A review on genotoxicity, its molecular mechanisms, regulatory testing in drug development process. *International Journal of Pharmaceutical Sciences and Research*, *10*(9), 4054- 4069.

Corvi, R., & Madia, F. (2017). In vitro genotoxicity testing–Can the performance be enhanced? *Food and Chemical Toxicology*, *106*, 600–608.

Vandghanooni, S., & Eskandani, M. (2011). Comet assay: A method to evaluate genotoxicity of nano-drug delivery system. *BioImpacts*, 1, 87–97.

Collins, A. R. (2004). The Comet Assay for DNA Damage and Repair Principles, Applications, and Limitations. *MOLECULAR BIOTECHNOLOGY* , 26, 249-261.

Merk, O., & Speit, G. (1998). Significance of Formaldehyde-Induced DNA-Protein Crosslinks for Mutagenesis. *Environmental and Molecular Mutagenesis*, 32, 260-268.

Speit, G., & Schmid, O. (2006). Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells. *Mutation Research - Reviews in Mutation Research*, 613, 1–9.

Özhan, G., & Alpertunga, B. (2003). Genotoxic Activities of Drug-Nitrite Interaction Products. *Drug and Chemical Toxicology*, *26*(4), 295–308.

Li, J.-H., & Lin, L.-F. (1998). Genetic toxicology of abused drugs: a brief review. *Mutagenesis*, 13, 557-565.

Yujra, V. Q., Moretti, E. G., Claudio, S. R., Silva, M. J. D., Oliveira, F. de, Oshima, C. T. F., & Ribeiro, D. A. (2016). Genotoxicity and mutagenicity induced by acute crack cocaine exposure in mice. *Drug and Chemical Toxicology*, *39*(4), 388–391.

Husgafvel-Pursiainen, K. (2004). Genotoxicity of environmental tobacco smoke: A review. In *Mutation Research - Reviews in Mutation Research*, 567, 427–445.

DeMarini, D. M. (2004). Genotoxicity of tobacco smoke and tobacco smoke condensate: A review. In *Mutation Research - Reviews in Mutation Research*, 567, 447–474.

Kotova, N., Vare, D., Schultz, N., Meesters, D. G., Stepnik, M., Grawé, J., Helleday, T., & Jenssen, D. (2013). Genotoxicity of alcohol is linked to DNA replication-associated damage and homologous recombination repair. *Carcinogenesis*, *34*(2), 325–330.

Bhide, S., Shiatapurkar, N. M., Gothoskar, S. v, & Ranadive, K. J. (1989). Carcinogenicity of betel quid ingredients: feeding mice with aqueous extract and the polyphenol fraction of betel nut. *Br. J. Cancer,* 40, 922-926.

Peters, M. F., Choy, A. L., Pin, C., Leishman, D. J., Moisan, A., Ewart, L., Guzzie-Peck, P. J., Sura, R., Keller, D. A., Scott, C. W., & Kolaja, K. L. (2020). Developing: In vitro assays to transform gastrointestinal safety assessment: Potential for microphysiological systems. *Lab on a Chip Royal Society of Chemistry,* 20, 1177–1190.

Pusztaszeri, M. P., Genta, R. M., & Cryer, B. L. (2007). Drug-induced injury in the gastrointestinal tract: Clinical and pathologic considerations. *Nature Clinical Practice Gastroenterology and Hepatology* , 4, 442–453.

Bergman, K., Müller, L., Teigen, S, W. (1996). The genotoxicity and carcinogenicity of paracetamol: a regulatory review. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 349, 263-288.

Dybing, E., Holme, J. A., Gordon, W. P., Soderlund, E. J., Dahlin, D. C., & Nelson, S. D. (1984). Genotoxicity studies with paracetamol. *Mutation Research*, 138, 21-32.

Rannug, U., Holme, J. A., Hongslo, J. K., & Srim ', R. (1995). Internatlonal commission for protection against environmental mutagens and carcinogens an evaluation of the genetic toxicity of paracetamol. *Mutation Research*, 327, 179-200.

Rina, T., Pankaj, T., & SS, P. (2014). Genotoxicity Studies of Diclofenac Sodium in the Bone Marrow and Germ cells of Laboratory Mice. *Austin journal of pharmacology and therapeutics,* 2, 1054.

Hartmann, A., Erkman, L., Maremanda, N., Elhajouji, A., & Martus, H. J. (2021). Comprehensive review of genotoxicity data for diclofenac. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, *866*.