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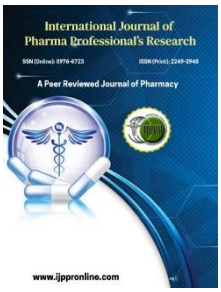

Unraveling Drug Safety: Importance of Toxicological Screening and Animal Models in Pharmacokinetics Studies for Clinical Medicinal Impact

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ABSTRACT:

The pharmacokinetics of a drug is an important factor in determining the safety of the drug. In this review, we focus on the importance of the toxicological screening and the methods using various animal models to determine any signs of toxicity in the drug substance. The toxicity data acquired is used to determine the relationship among the serum composition and the toxicity results, as well as their purpose and importance to the drug's clinical medicinal impacts. *in silico* models which generally utilize the help of computers study the parameters of ADME in an artificial environment and generally costlier model i.e., animal model is used in the later stages of testing of medications potency and hazards. *In vitro* studies are conducted in rats and are conducted firstly to offer data for different regions of body favoring pharmacological investigations. The radiation levels determined by quantitative whole-body autoradiography amongst various tissues at different point of time are important to study that why some compounds fail to exhibit favorable PK during preclinical ADME screening, this information could be immensely helpful to medicinal chemists for improvement of the weak backbones. Preclinical studies are modest, but they can become better if all the test procedure are followed in the right manner and right evidence is provided which highlights the desired biological effect at the same time risk assessment methods should also take place in case of any severe adverse effects.

Introduction:

The word toxicology is derived from the Latin and is a combination of two words toxico and logy which means study of toxins and poisons with their potential effects and treatment.[1] A drug must first undergo various stages of screening and trials before entering the market. If the potential drug candidate has passed all the tests, then the manufacturer can apply to FDA for their approval. The purpose of the preliminary investigation should be to evaluate the toxic potential of the active substance following both acute and chronic exposure. We must guarantee that the medicine is harmless and benign on the research subjects before proceeding to the first step of the clinical study.[2] Pre-formulation is the process of determining the molecular and structural characteristics of a drug component and designing a drug molecule, based on those attributes the amount of dose to be given and the route of administration is determined. However, the very first step always is to ensure that the drug molecule under the observation is safe and free from the toxicity. It is a belief that animalia modeling are highly indicative of treatment outcome in humans and it provides the base for implementing the toxicity testing in the research to establish a safe and effective drug which will eventually cure the human diseases with minimal side effects. The use of experimental animals varies from country to country, there have been instances of numerous governments neglecting to document or disclose statistics on exploitation of experimental animals during animal model studies. Those countries that are advanced in using animal-related models only record data on animals that survive the test and fail to collect information on animals that are sacrificed due to disease or the collection of some of the organs from the experimental animals.[3] Hence, we can say that it is humanly not possible to record all the data related to the animal used for research and toxicological testing. To overcome this and many other problems related to animal model we are now shifting towards *in-silico* modelling about which we will be discussing in this paper. This review focuses on the importance of toxicological screening and the methods using various animal models to determine any signs of toxicity in the drug substance.

1.1 Sources of Toxic Substances

Toxic substances are often categorized according to their molecular composition, mechanism of action, or its category. The toxins are divided according to whether they are found in diet, atmosphere, water, or soil or the toxicants can be categorized according to their usage as substances of abuse, medicinal medications, agrochemicals, dietary supplements, and insecticides.[4] **Table 1** list the various sources of toxicity. Before conducting any toxicological testing in animals, the reviews must first be presented to and authorized from the Institute of Animals Ethics Committee, or the procedure followed by the manufacturer should meet the standards of the local regulatory agency.[5] The Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA] throughout India establishes all the criteria's that must be fulfilled for the proper care of experimental animals. Schedule Y contains the list of all the requirements related to regulation.[6]

Table 1: Toxicological sources in drug development.

Type of pollutant	Sources	Examples	References
Air pollutant	Natural Pollutant, Anthropogenic and Indoor Pollutants	Carbon monoxide, Sulfur Oxides, Nitrogen oxides, Arsenic.	[4]

Water and soil pollutant	An industrial plant's effluent pipe, rainwater transports insecticides and fertilisers from a farm into a river, Household, and city waste	Lead, Arsenic, Cadmium, Mercury.	[5]
Occupational pollutant	Dermal and inhalation are the most common routes of industrial exposure. If meals or drinkable water is polluted, poisonous entities may be ingested.	Aniline, Carbon tetrachloride, Cadmium	[4]
Synthetic pollutant	Cadmium, Lead and Mercury	Materials for semiconducting chips and transistors, gaskets and windows, and laminated circuit boards	[7]
Domestic pollutant	These are present in the household as waste material.	Substances from the construction spot, used cooking oil, and other domestic chemicals are all considered waste.	[8]

1.2 Toxicokinetic

Study of toxicity is an integral part of non-clinical toxicity assessments in determining systemic vulnerability of the dose. The animal toxicological evaluation is the cornerstone of the Investigative New Drug meanwhile toxicokinetics is an essential component thereof.[9] Pharmacokinetics and organ dissemination are conducted on a satellite set of animals. To determine and compare the results with the interpretation of toxicological findings in the animals is called toxicokinetics. Pharmacokinetics is very much contrasting to toxicokinetics as the former criteria are used for medication description, whilst the latter is used to quantify the systematic exposition of the medication attained in animals at multiple stages of dosage provided after concurrent dosing of the drug.[10] The pharmacokinetics data acquired is used to determine the relationship among the serum composition and the toxicity results, as well as their purpose and importance to the drug's clinical medicinal impacts. The Toxicokinetic data, together with the toxicity results, aids in the selection of toxicity species and the subsequent planning of non-clinical toxicity studies.[11] Even though the main goal of the toxicokinetics is always to detect the blood and tissues thresholds of the active ingredient in, there seem to be situations when the intermediate quantity in blood or different bodily fluid could prove crucial for toxicokinetic study completion.

1.3 Toxicology Studies

It is impossible to discuss toxicity research within the framework of medication development without bringing pharmacological activity and pharmacokinetic studies into account. Such critical areas provide information not only for creating medicine in general, as well as for toxicity investigation in specific. A drug's pharmacological evaluation material arises when relevant parameters are assessed. The results of these exploratory tests frequently serve as the foundation for clinical testing while also assisting the experts for evaluating their structure and its application.[12] These investigations are designed to evaluate the frequency and degree of absorption, enzymatic pathways, frequency of

excretion from the system & anatomical distributions following single and multiple dosages of the medicine. In terms of metabolic patterns, it is not necessary to identify every metabolite in the early stages. The goal of toxicology research is to identify possible negative effects in animals after medication delivery for varied time periods. The intend should be to reach the primary objective of creating wisdom by striving to adequately identify operational and structural consequences in animals, that will aid in the decision of whether the treatment should be explored in people or not. More crucially, the data will forewarn of possible human dangers if the medicine is put through clinical trials.[13] Toxicology studies must fulfil the criteria of the companies, medical researchers, pre-clinical investigators, and worldwide legislative authorities, and they ought to be carried out in compliance with approved laboratories regulations in different countries. Various governmental authorities and health organizations have given guidelines tailored to local needs. The recommendations specify the basic procedure for conducting research as well as the minimal requirements acceptable to the various nations and organizations. Guidelines are often sufficient to offer investigators the imagination and versatility required to construct research tailored to a specific category of medicine or drug use.[14] Once it is realized that one may expect the lethality of a new treatment to just be comparable with the existing medications, such flexibility and inventiveness become highly crucial. It is consequently critical that each medicine be investigated as a distinct and unique entity. The period of animal toxicology research is influenced by the expected length of clinical trials.

1.4 Need For Preclinical Studies

Preclinical testing of a drug is performed to determine whether the potential drug candidate has met all of the basic requirements for human studies. This study aids in the establishment of dosage and response in prediction of pharmacology and the hazardous consequences associated with it. Distribution of the medication to the organs and identifying metabolic, kinetic, and elimination pathways of the drug is also noted.[15] Preclinical testing is classified into two categories: (1) Acute toxicity testing – Short term animal studies, (2) Chronic toxicity testing - long term animal studies. After this data is collected and if the drug candidate passes all the stages, then the report is sent to FDA for IND filing. The preclinical studies are generally performed to gather the basic information regarding the stability and efficiency of the medication before it passes for further studies in human models.[16]

1.5 Dose Selection and Route of Administration:

It is commonly observed that high doses cause toxicity, but this is not always the case. High doses are determined based on food consumption, alternation in chemistry, maximum tolerated dose, and maximum high dose. High doses are determined by the criteria which are unaffected by technological or physical and chemical factors.[17] The maximum systematic dose should be considered as the high dose. **Table 2** illustrates various route of administration.

Table 2: Common administration routes:

Route of administrations	Adequate range of volume	Advantages	Disadvantages	References
Oral (capsule)	2 g/kg	Can be easily administered in dogs, it can be easily tolerated after some	Due to excessive first pass metabolism, bioavailability is limited.	[18,19]

		time of the exposure.		
Oral (food or drugs)	5 mL/kg	This method is easy to administer mostly in all the common species, different vehicles used in formulations can be given by this route.	Due to excessive first pass metabolism, bioavailability is limited.	[20]
I.V.	Up to 5 mL/kg	This method is easy to administer mostly in all the common species, different vehicles used in formulations can be given by this route	Uses high quality sterile preparation and its administration techniques, the volume of drug present at the site of injection being applied,	[21]
I.M	Up to of 0.05 mL/kg per animal site of admini	This method is easy to administer mostly in all the	Uses high-quality sterile preparation and administration	[21,22]
	stration.	common species, different vehicles used in formulations can be given by this route.	techniques, with the volume of drug present at the injection site, tolerance could be developed in the experimental animal.	
S.C.	Up to of 5 mL/kg animal site of administration.	This method is easy to administer mostly in all the common species, different vehicles used in formulations can be given by this route.	Uses high quality sterile preparation and its administration techniques, tolerance could be developed in the experimental animal.	[22]
Intra Dermal	0.05-0.1 mL per site of administration.	Most preferred route in guinea pig, systemic exposure of the drug is less.	Highly skilled personnel are required to carry out the test, to prevent any tolerance related	[18]

			problems.	
Intra Nasal	At least 35µL per experimental animal.	Route of administration is preferred as it is a noninvasive technique.	Not all medications can be instilled through nasal cavity.	[23]
Inhalation	5 mL/kg for 10 minutes or 10 mL/kg for 5 minutes.	It avoids first-pass metabolism, which reduces systemic adverse effects; it is painless, which increases patient compliance; and it delivers the medication directly to the site of action, which results in a rapid onset. Most drugs that are inhaled have their therapeutic effect in the lungs.	Irritating effects on the airways. extended use time, it is necessary to have good respiratory coordination.	[24]

3. Common Species Used for Toxicity Testing

Animals are subjected to toxicity tests so that we can learn more about a substance's potential side effects and establish dose-response relationships that will allow us to evaluate reactions at different doses. Careful design has reduced the possibility of both false-positive and false-negative outcomes in toxicity testing. These targets, however, are evaluated in the context of limited funds and other assets. When planning toxicity studies for human health risk assessment, it is critical to consider the variety and level of human exposure. Knowing the chemical stability of the substance and any potential breakdown products, as well as the length, frequency, intensity, and routes of exposure, can all help with dosing regimen, test medium, and test material selection.[25]

Table 3 shows various species which are used for toxicity testing while **figure 1** depicts the various routes used to provide a dosage to a rat.

Table 3: Animal species used in toxicity testing.

ANIMAL SPECIES	**ADVANTAGES**	**DISADVANTAGES**	**REFERENCES**
Mouse	It uses a smaller number of test animals, with various route of administration possible, several transgenic mouses are available.	Blood volume is generally limited.	[26]

Rat	Less number of experimental rats are used, generally they are easy to handle, and monitoring is easy.	Blood volume is limited.	[27]
Rabbit	There is a close sensitivity in the dermal model with humans.	Mortality rate is higher when stress is applied	[27]
Guinea pig	They are most preferred choice for dermal model, now they are also being used for oral route of administration.	Large number of test animals are used during studies, highly skilled personnel are required, only selective route of administration is possible.	[26]
Monkey	Limited experimental animals are required, different route of exposures is possible. This model has higher relevance with human beings.	Highly skilled personnel are required to carry out the test, housing of the animals should be specialized.	[28]
Dog	Different route of exposure is possible, comparatively easier to handle than others.	Fail to accurately represent the human body or the behavior of human diseases in response to medications, chemicals, or treatments.	[27]
Chimpanzees	Relatively close to human people. Generally used in fertility and toxicity study. All facets of reproduction carcinogenicity should	The ethical quandaries involving their utilization in laboratory research, combined with	[29]

	be researched within the same species.	their loss of habitat, are indeed the major limitations preventing the use of this species.	
Zebrafish	Due to its clarity, low price, transgenic and its capabilities to modify gene expressions, preservation of cell signaling, and correspondence with mammalian neurodevelopment phenotypes, this small model is useful for studying vertebrate development.	Physical barriers dissolution rate, chorionation), kinetic factors such as uptake, transport, metabolism, elimination, and dynamic factors including sensitivity and sensibility are all obvious limitations of the zebrafish for chemical testing	[30]

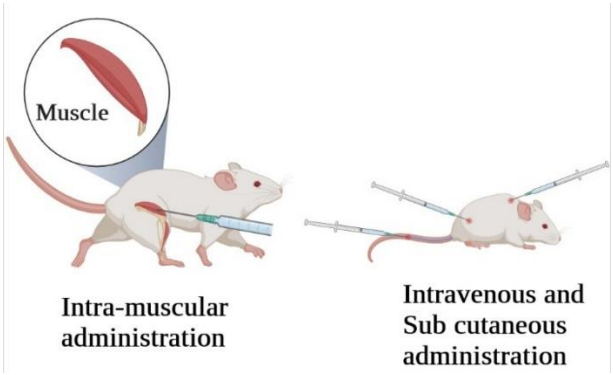

Figure 1: This image shows how the drug can be introduced in the rat's body through different routes.

4. Toxicological Testing

Toxicology testing, also known as safety assessment or toxicity testing, is the process of determining the extent to which an organism's normal biological functions are disrupted by an exposure to a substance of interest, given a specific exposure duration, route of exposure, and substance concentration.[31] Researchers frequently conduct toxicological testing in accordance with established toxicology test procedures for a given drug, mode of exposure, exposure environment, length of exposure, organism of interest, or developmental stage. Toxicology testing for a chemical that will be used in humans is typically performed during preclinical development.

This report in **table 4** provides toxicity studies data in support of clinical trials conducted in the UK, USA, and Japan.

Table 4: Duration for toxicological testing.[19]

Time Frame for experimental tests	**Time Frame for Recurrent Dosage**	**Time Frame for Recurrent Dosage**

	Toxicology Studies in Rodents	**Toxicology Studies in Nonrodents**
Solitary dose	14 to 28 days	14 days
For 14 days	14 to 28 days	28 days
For 30 days	30 days	30 days
For 90 days	90 days	90 days
For 180 days	180 days	180 days
More than 180 days	180 days	Chronic

4.1 Acute Toxicological Testing

Acute toxicity pertains to the undesirable consequences that occur after a single dosage of a drug is given orally or through dermally as well as multiple doses being provided within 24 hours or acute toxicity can also be due to inhalation exposure for up to 4 hours.[32] Acute toxicity assessment is usually performed to observe how a single dosage affects the test species of animals.[33] Traditionally, information on acute toxicity has been gathered by carrying the study in two different mammalian species (typically, one animal from rodent species is selected and one from and one nonrodent is chosen) utilizing both clinical and intravenous modes for drug delivery. The test drug is given at various dose levels, and their effect is monitored for up to 14 days.[34] Information obtained after this study of acute toxicity testing of drugs could help us predict the consequences in case of overdose situation in human beings and this data should be available to support phase 3 trials. At the conclusion of the research, experimental animal is sacrificed while the tissues are extracted for further evaluation. Acute studies of this sort could help to justify micro dosing in people by accumulating initial data on pharmacological, pharmacokinetics, and mode of action.[35]

4.2 Repeated Dose Toxicity

This test is conducted over a minimum of 28 days. The possible pharmaceutical drug is delivered through the oral route for a certain period. If this route is not considered as convenient depending upon the status of the animal model the test drug can also be administered through the parenteral route. There should be little or no variations between the animals. Throughout the test phase any behavioral or pharmacological parameters changes are noted.[36] Tissues from most of the organs are taken after the conclusion of the experiment, and any alterations are documented.[37] This interpretation of the test results is extremely helpful in determining human safety due to repeated exposure to a toxic substance.[38]

4.3 Sub-Acute Toxicological Study

Typically, a 2-week investigation is adequate to support a phase 1 clinical trial. Although on some clinical indications this is enough however, for most products, a 4-week study is required to give adequate safety evidence for clinical trials. This 4-week study is carried out to follow up the study after the acute dose.[39] Therefore, this four-week research is being conducted to improve the product's toxicity profile data. Procedure for carrying out this test is same as the acute dose testing, but this is helpful to determine the toxicity after repeated exposure.[40]

4.4 Subchronic Toxicity Testing

This testing is performed on one animal from each of a rodent and one of a non-rodent. During rodent investigations, around 25 rodents per sex were used from every batch.[34,38] A non-rodent species (generally monkeys) is also taken because of their similarities to humans. The medication is administered to the test animals lasting up to 13 weeks and any signs of toxicity is observed. Toxicokinetic sampling is performed at the start, middle, and end of the dose cycle. During the end of the study, it is necessary to conduct carcinogenicity evaluations in the animal species. The animals are sacrificed after the conclusion of the research.[41] This 13-week test study is

necessary for the dosages to be considered in the oncogenic evaluations. The information obtained is generally identical as in multiple dosage toxicity, however the goal is to determine a high dosage that is clinically effective during the exposure time instead of completely defining the toxicity characteristics.[42]

4.5 Chronic Toxicological Testing

Generally, countries like USA and Japan conducted chronic toxicity testing for 12 months and the EU agencies conducted it for 6 months.[43] Due to this difference many pharmaceutical companies were forced to conduct two chronic repetitive dosage experiments in rodents and non-rodents, one for 180 days to assist clinical trials and the other for 365 days to assist commercialization in the United States and Japan. Following long evaluations ICH proposed the maximal length of long-term repeated dosage toxicity in rats should be decreased from 365 days to 180 days, and in non-rodent species from 365 days to 270 days.[44] Chronic testing is conducted beyond the span of 90 days, meanwhile the animals are monitored on a regular basis. There should be little or no variations between the animals. This toxicity research tends to give details regarding the long-term impacts of the experimental drug on animals, therefore such findings may be extended to human safety after consumption of the experimental drug.[45] While conducting the research, the animals' regular functions are examined, their characteristics are recorded, and any alterations in biochemical processes are noted. After the research is concluded the animals are sacrificed while tissue specimens from all organs are retrieved for histological investigation.

4.6 Oncogenicity Studies

Rodent and non-rodent species can both be used for oncogenicity.[46] The examination is performed over a prolonged span of time, usually throughout the animal's life. The experimental animal is first observed before giving any test substance and further the animal is also observed during and after the exposure of the test substance for development of any type of toxicity or development of tumours.[47] If no toxicity is found the test can be ceased after one and a half year in the instance of rodents and in the instance of hamsters it is carried for 2 years. If the animal is healthy, cellular components of the blood is analysed after 12 months and again after 18 months, if everything seems fine the research is then sought to be completed. After the research is completed, the animals are sacrificed and pathognomonic symptoms and histological analyses on the tissues are conducted.[48]

4.7 Neurotoxicity Studies

The investigation of the synthetic, pharmacological, and physical risks on the central nervous system and on behavioural patterns throughout growth & maturation is known as neurotoxicology.[49] The topic of risk assessment in cancer and other various diseases end points, including neurotoxicology, has garnered tremendous interest, as a consequence of which a structured investigation and administration paradigm for assessing danger linked to either physical or chemical elements' exposure has been developed.[50] The effect of a test substance on the CNS can be studied through the study using rodents as the test animal. The PNS is segregated into two parts: the somatic nervous system and the autonomic nervous system.[51] Neurotoxicity screening is used to assess a chemical's histopathology as well as cognitive neurotoxicity and to retrieve neurotoxic signals such as lesions, memory loss, sensory abnormalities, memory and recall dysfunctions. Neurotoxicity investigations are often conducted in mature rodents. The sample is given for 2 weeks, and in certain situations, it is given for more than 3 months.[42] The neuronal alterations are indeed assessed.

These methods are extremely useful in managing the likelihood of unfavourable responses by detecting the problems associated with the molecular properties of the development compounds. If this approach is applied at in the initial phases of medication discovery, this will aid in the identification of drug candidates with limited risks, hence enhancing the quality of the production cycle by reducing the possibility of unfavourable characteristics. These findings are in combination with the preliminary and therapeutic safety evaluations of the novel medicinal compounds, will aid in risk evaluation and the creation of acceptable and useful medication prospects for market usage.[52]

5. Biopharmaceutical Safety Evaluations

When the pharmaceutical component is a biological protein or a large molecule, biotechnology-derived molecule, preclinical toxicity studies are challenging, especially the duration for the chronic investigations. This is because of these chemicals' ability to target humans specifically. Potential assessment concerns include as, (i): In rodents or pups, biological proteins or receptors may not be biologically effective,[53] (ii): In such animal species, the treatment medication may be so allergenic that lengthy studies are not possible due to the production of neutralising anti-bodies. Choosing a pertinent species of animals to be used in toxicology research is therefore difficult for preclinical safety assessment of biopharmaceuticals. The most critical aspect in species selection is that the medicine should be pharmacologically effective in the species under investigation.[54] This is a key factor since biologics is designed to tackle functional classes and rarely induce off-target effects. Primates other than human beings are often the only pertinent species that can be used to assess the safety of biologics.[55]

6. ADME Screening

6.1 *In-Vivo* ADME Screening

Previously, obtaining a desired pre-clinical phase was not required to develop a comprehensive pharmacokinetic characterization of the chemical of concern; instead, a ranking sequence was thought to be sufficient.[56] If the earlier models are followed at this point, the rate at which we find and reject molecules will determine the pace of the process of medicinal discovery. The applicants with an unfavourable toxicological property will indeed be judged as minimal or of zero significance, would be eliminated from study and would enable the scientist to focus more on the potential lead compounds to yield better results. There are many in-vivo screening methods to determine the active and weak compounds according to the interest of the scientists. These methods are cassette dosing,[57,58] cassette analysis[59,60] and rapid rat screening,[61] that had already been devised for immediately assessing applicants. We believe that in the future direct PK profiling in the humans would significantly reduce the use of animals and would also save time of the researcher which would yield in discovery of the drug candidates at a rapid pace.[62,63]

(A) **Cassette Dosing**: It is a method for assessment of maximum capacity of drug candidates in product development that will result in a quick assessment of the pharmacokinetic profile of a significant quantity of pharmaceutical prospects. It has been used for long to mark the compounds depending on oral serum concentrations or the drug's systematic elimination following an IV dose.[64] This feature of the screen has enabled in high efficiency, has reduced the animal usage and a single report can be generated for several different compounds. This technique has not been able to gain enough acceptance in the market due to several different practical difficulties that are associated with the screen. These difficulties that occur are a false

positive result which means that the compound does not have a suitable PK profile, but the screen shows otherwise and vice versa i.e., a compound with favourable PK profile is shown as unacceptable in the design cassette this is called as false negative.[65] Often the false negative results are deemed more dangerous than false positive results since promising compounds might be discarded without further verification.

(B**) Cassette Analysis**: This method is frequently used as a substitute to cassette dosing. In this procedure we mix comparable quantities of serum taken at different time periods from already medicated animals.[66] This approach does have the benefit of producing consistent findings than cassette dosing methods since it does not produce any false negative or false positive outcomes. The time to analysis is often reduced more significantly with the direct injection of the drug or switching the column. Nonetheless the usage of animals and differences amongst animals however are hardly minimised.

(C) **Rapid Rat Screen Model**: In this method a particular chemical substance is given to both the rodents, then plasma was taken at particular intervals of time from each rodent for 6 hours. After that equal amount of plasma derived from the rats at equal amount of time is then grouped and analysed using a standard curve.[67] This study helps to gain knowledge about the molecules ½ life. The rapid rat screen method is devoid of drug - drug interactions and such a method are easy for analysing single compound and the time to analysis is also relatively short as there is need of only four samples per study. However, the limitations associated with such a study is since the area under the curve and ½ life derived from the experiments simply represents approximations rather than genuine true values, therefore there is no way to determine the maximum time or maximum concentrations. Such techniques are helpful for screening of drug candidates for preliminary identification of promising compounds with the appropriate pharmacokinetic attributes for investigation via a comprehensive pharmacokinetic testing.

(D) **Complete Pharmacokinetic Screening:** If PK of identified substances is needed for repeated testing, it could be conducted on a substantial amount of laboratory experimental for example, rodents, rats, mice or pig by providing a single dose of the chemical to confirm the findings of *in vivo* pharmacokinetic assays and create reliable pharmacokinetic data.[10] Such research is especially useful in detecting *in vivo* intermediates, which might be tough to recognise in a tangled chain of multiple substances. The compounds that satisfy the *in vivo* screening can subsequently be exposed to further *in vitro* experiments to establish the pace and degree of degradation amongst multiple animals to forecast the response amongst humans**. Figure 2** shows the progression of the drug discovery process.

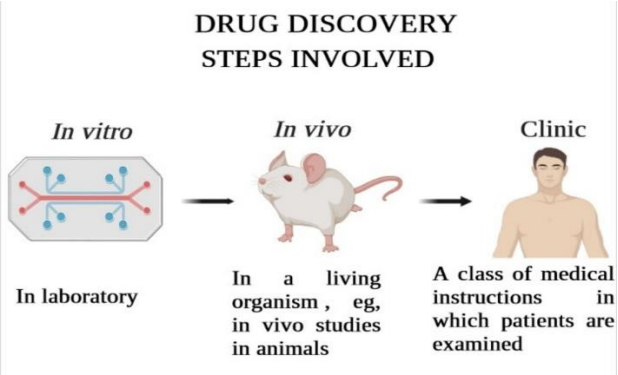

Figure 2: Steps involved in drug discovery

6.2 *In-Vitro* Screening

In vitro study helps the researchers in extending the *in vivo* prototype of pharmacokinetic characteristics amongst humans to efficiently communicate information regarding the differences that occur in distinct species which is coined as interspecies differences. Recombinant mammalian cytochrome p450 enzyme and hepatic microsomes seems to be the assessment technologies which are being mostly preferred as

they are readily available.[68] The disadvantage associated with this type of study method has been a concern that these cannot accurately depict the biological background. The challenges associated with the *in vitro* screening is particularly important to study that why some compounds fail to exhibit favourable PK during pre-clinical ADME screening, therefore this information could be immensely helpful to medicinal chemists for improvement of the weak backbones.[69] For e.g., To recognise the reasons of poor bioavailability of orally administered drugs, one must investigate the possibility of poor dissolution, minimal permeation, and turbulence in stomach and gastrointestinal fluids. If everything looks to be in order, the most probable cause is either of the mentioned: (i) The serum concentrations are sufficient; however, the substance is ineffective - It might happen for chemicals having a short ½ life and a rapid elimination rate. (ii) Although the serum concentrations are less, still the substance is effective—this suggests that the molecule has physiologically converted itself into its bioactive intermediates.[10]

7. Limitations of Animal Studies in Predicting Toxicity

The most important objective is to design a safe and effective dosage form of a drug. Due to the negative side effects related to toxicity there are many drugs that reach the stage of clinical trial, but they eventually fail to reach to the market, the main reason behind this is that not all the animal models are able to replicate the exact same conditions that are required in humans.[70] Drug passes through various stages of trials and after passing every stage then only the drug is allowed to enter the market. There is no denying that using animals in research and medicine has benefited people.[71] However, a large number of researchers are concerned because animal experimentation could be based on a factually flawed facility, given that their continued acceptability is only conceivable because no clear alternatives have been discovered. Many people are reconsidering the value of animal studies in the drug development process, owing to rising costs and unusually high failure rates. Even when performed in compliance to strict protocols, replicability with animal likeness within species is debatable. A study discovered that toxicity was only repeatable in the same species 70% of the time that used a dataset with over eight lakh animal toxicological tests completed under precise standards for three hundred and fifty substances.[72] Another study discovered that the outcomes for a single chemical varied depending on the given animal paradigm, breed, dose quantity as well as method of administration. Due to this vigorous testing of drug at every stage, it has been noted in the studies that animal models fail to predict the toxicity of close to 50% of the drugs that are stationed between Phase 1 of clinical trial till the marketing phase.[73] So, we always want quality testing that predicts the drug toxicity at early stages of the studies as it will not only reduce the cost but will also help to track back to the step which will lead to the process which caused to toxicity. This will eventually save time as the whole process of drug testing and studies is naturally a long process.

7.1 Alternative Advanced Methods for Preclinical Studies

Despite significant effort and money placed on what might out to become a therapeutically failure as clinical animal studies struggle to predict toxicity for approximately 50 percent of drugs throughout the pathway across phase I trials and early post market abolitions.[74] Novel medication screening techniques that yield more accurate, timely, and easy - to - interpret data will boost human welfare.

8. *In Silico* Modelling - Timely assessment of toxicity that might occur in human beings is crucial for minimising medication production expenses, and now computational analysis has subsequently been promoted as an essential,

sapient preclinical evaluation tool.[75] Many of most notable advantages of computational analysis is in regard to the amount of chemicals which can be examined rapidly, and variety of scenarios can be quickly simulated. One more potentially important benefit of *in silico* evaluation is its capability to identify areas for rescuing and repurposing of the drugs.[76]

Models For Drug Development

8.1 Adme *In Silico* Models

ADME stands for absorption, distribution, metabolism, & excretion. ADME is an important process which we try to predict of a new drug molecule which are generally a result of all the physiological processes.[77] These parameters hold a vital cog in determining the amount of dose, dosage interval and overall safety margins. To optimize the parameters of ADME there are several different *in vivo* and *in vitro* techniques which are generally employed for screening of a new drug, these models which are used are cheaper and provide results with high efficiency throughout the process meanwhile the costlier model i.e., animal model is used in the later stages of testing of medications potency and hazards.[78] The proportion of pharmaceutical compounds being tested in this experimental model have expanded dramatically in recent years which has resulted in development of *in silico* models.[79] *In silico* models which generally utilizes the help of computers, study the parameters of ADME in an artificial environment with the help of computers that helps to assess the molecule's toxicity and potency. This model is also used widely because it reduces the cost expenses and save time as formulating a new drug molecule is a very time-consuming process.[80] With the knowledge of all the ADME parameters one can determined the Minimum Effective Concentration [MEC] at which drug will produce its therapeutic action and Maximum Tolerated Dose [MTD] which helps to determine and prevent any undesirable side effects.[81]

8.2 Pre-Clinical ADME Models

Researchers should always conduct a mass balance and ADME studies during drug discovery, that are carried out using non-radiolabeled substances which yields little quantifiable values of both parent compound and its derivatives. Pre-formulation investigations are carried out during therapeutic formulation using typically 14C or 3H marked substances that offer more specific insight about the circulating constituents.[82] Tissue distribution studies are performed in rats and are conducted to offer data for different regions of body favoring pharmacodynamic investigations. Such investigations are confined to a solitary dosage which is administered through the chosen method of delivery, usually via PO. (i.e., oral administration) or IV (when a substance is given through veins) following the completion of the research, the test animals are sacrificed while their tissues and organs are extracted out for further evaluation. The radiation levels is determined by (QWBA) quantitative whole-body autoradiography amongst various tissues at different point of time.[83] In this process the whole section of the animal body is given exposure to the phoshorimaging screen and then they are scanned with the help of a phosphorus imaging system. The radioactive data is projected into living tissues depending on the rodent's bodily surface size and weight.[84] Tissue distribution studies has its own application for instance, considering the assessment of mother to fetus transfer. Such investigations are necessary in filling for NDA (New Drug Application) as well as INDA (Investigational New Drug Application). The species which have been employed with the mass balance investigations, are selected depending upon determining products extended toxicity assessment. The amount of radioactive material that is administered in the rat is governed by the source products pharmacokinetic characteristics. Typically, the spectrum of delivery is between 1.5 to 100 micro-Ci/kg.[85] The duration of study is

generally done for more than five serum 1⁄2 lives. Drug products which have extended 1⁄2 lives, their studies are conducted for more than one percent of the overall dosage eliminated till 24h interval.

8.3 Forecasting Toxicity Using *In Silico* Stimulations

Use of computer modelling in predicting the pharmacological objectives have been especially demanding due to the speed with which the data are delivered with minimal expenses.[86] These techniques can be used during the beginning stage of the drug discovery even before the molecule has been synthesised. There are numerous available computer software's which are free-web programmes used for prediction of toxicity, e.g., DEREK for Windows, MCASE, TOPKAT.[87] In the market, computational simulation forecasting systems is categorised as 'intelligent machines' and 'research-based systems'.[88] Although the majority of these systems provide specificity, 80% of those that suffer from moderate specificity (overall percentage of accurate positives + incorrect positives) are frequently in the 50% range.[89]

Data driven systems is commonly used for prediction of compounds which have comparable designs exhibiting the hazardous impacts.[90] Since several years, information driven SARs have shown excellent potential in the endeavour to forecast mutagenic characteristics and symptoms of chemical genotoxicity.[91] A variety of strategies, like partial least square method, recurrent partitioning and multiple linear regression are being used.[92] Apart from selecting suitable algorithm, the other major challenge is selection of a suitable physiochemical descriptor for developing QSAR.[93] Other than correctly predicting, another important task is the determination of the pharmacological properties responsible for the reported toxicity.[94]

8.4 *In Silico* Modelling

This type of modelling has been widely in use nowadays, as it set the bases for evaluation of human based model in pre-clinical evaluation. We have discussed already that the early prediction of toxicity in pre-clinical studies is essential as it will decrease the cost of drug development.[95] *In silico* modelling can also be referred to as computational modelling in which computers are used to stimulate the human conditions in an artificial environment.[96] One of the most significant advantages of using these models is the ability to evaluate a wide range of substances in a short period of time. However, computers cannot completely replace animal models in medical research, but it can significantly reduce the expenses in animal testing by giving satisfactory results from a smaller number of experimented animals which will automatically minimise the duration it takes for a fresh medication to reach the market and will result in overall reductions in cost expenses in drug development.[97] There are two major terms which are associated with *in silico* modelling they are:

8.4.1 Rescue

It is possible for a pharmaceutical drug to be successfully reintroduced into clinical trials for another use, provided that the drug has an effect that is comparable to that of the original application.[98] This situation is known as "medication formulations that were put through efficacy testing for one application but were proven to be unsuccessful." It is claimed that "one of the purposes of the study was rescued" when a drug candidate is successful in testing for a different purpose after it was unsuccessful in testing for the first purpose It is possible for a pharmaceutical medicine to be successfully reintroduced into clinical trials for another purpose.[99]

8.4.2 Repurposing

When an existing medication is used for a purpose that was not initially envisaged for it, this process is referred to as "drug repurposing", "drug

repositioning," or "drug re-profiling". It is possible that the drugs that were discovered in the past are now in use in clinical settings, even if it is for a different purpose than what was originally intended. Alternately, it is possible that these drugs have been "resigned" from any further progression due to an inadequacy of efficacy, a fear of negative impacts, or factors unrelated to scientific research, such as concerns regarding commercialization or marketing. In this context, "reapplying" a drug means putting it back into use after it has previously been tested in a clinical trial or after it has been removed from the market because a newer, more productive, and more effective drug has been developed.[100]

In both the above scenarios the preclinical and animals study phase could be avoided together even when there is a need of human trial phase, they may be conducted in a later phase study for some new indications which were not obtained during the previous studies.[101] This entire process could safe many years of vigorous testing. There are many researchers who have determined the estimated cost for rescue and repurposing, which is estimated to be about 40 million dollars to 80 million dollars as compared to approx. 1 billion dollar which is spent to formulate an entirely new drug molecule.[102]

9. Conclusion

The goal of performing preclinical study should be to ensure that the experimental drug ingredient produces the required results upon the investigations so to provide a suitable drug compound for the treatment of patients. We must also identify and characterize all the toxicities associated to the test drug and to predict any adverse effects in humans. Currently the outcomes of preclinical studies are modest, but they can become better if all the test procedure are followed in the right manner and right evidence is provided which highlights the desired biological effect of the drug at the same time risk assessment methods should also take place in case of any severe adverse effects. We have discussed methods that are used in pre-formulation studies but in all the methods the objective remains the same, i.e., to formulate an elegant, stable, safe, and effective dosage form. All this can be achieved by establishing kinetic rate profile of the drug molecule, drug excipient interactions and its compatibility. The major challenge however is accurately predicting the pharmaceutical products toxicity amongst the humans. The inability to forecast toxicity needs to be overcome. This failure is reported as a result of the drug's post-marketing withdrawal or new safety market labels that must be attached to the marketed drug label. There is a general belief among the companies that the significant funds and efforts devoted for characterization of the toxicity will compensate the companies by its benefits with high rate of success, which will eventually result in low rate of drug withdrawal from the market.

10. Conflicts of Ethics: Were declared zero.

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