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Design of Animal Experiments in Pharmacological Research

Farmakolojik Araştırmalarda Hayvan Deneyleri Tasarımı

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ABSTRACT

Pharmacology, also known as pharmaceutical science, has made significant progress, especially in the 20th century, and has played a fundamental role in the development of today's modern drugs. Pharmacology uses in vitro, in vivo, and clinical research stages in drug development. Experimental animals are of great importance in in vivo research. The majority of the drugs used today were developed thanks to animal research. Research in which experimental animals will be used should be planned carefully, and a minimum number of animals should be used since the subject is a living being. In addition, one of the most important ethical principles is to avoid procedures that may cause unnecessary torture and pain to animals during experiments. The purpose of pharmacological research is to develop drugs for the treatment or diagnosis of diseases. For this reason, it is aimed at determining the effects of the substance you are researching in the presence of disease. Immediate use of a substance whose effects were previously unknown on humans may lead to various adverse events and even death. After many events in the past, drug development stages have been determined by accepted international rules. According to these rules, the effect of the substance being investigated must be investigated in experimental animals that have been used as disease models before humans. Many disease models have been developed for this purpose. Drugs developed in these disease models created in experimental animals are now successfully used in the treatment of humans.
Keywords: Pharmacology; experimental animal; disease models; medicine; experimental design.

ÖZ

İlaç bilimi olarak adlandırılan farmakoloji özellikle 20. yüzyılda çok önemli bir ilerleme kaydederek günümüz modern ilaçlarının geliştirilmesinde temel rol almıştır. Farmakoloji ilaç gelişiminde in vitro, in vivo ve klinik araştırma basamaklarından yararlanmaktadır. Bunların içerisinde bulunan in vivo araştırmalarda ise deney hayvanlarının önemi büyüktür. Günümüzde kullanılan ilaçların büyük çoğunluğu deney hayvanları araştırmaları sayesinde geliştirilmişlerdir. Deney hayvanlarının kullanılacağı araştırmalar, öznenin canlı bir varlık olması nedeniyle dikkatli planlanmalı ve asgari sayıda hayvan kullanımı sağlanmalıdır. Bunun yanı sıra deneyler sırasında da hayvanlara gereksiz yere eziyet ve acı verebilecek işlemlerden kaçınılması en önemli etik ilkelerdendir. Farmakolojik araştırmaların amacı hastalıklara karşı tedavi veya tanı amacıyla ilaç geliştirmektir. Bu nedenle araştırılan maddenin hastalık varlığında etkilerini tespit etmek amaçlanmaktadır. Daha önce insanlarda etkileri bilinmeyen bir maddenin hemen insanlarda kullanılması çeşitli olumsuzluklara hatta ölümlere yol açabilecektir. Geçmişte yaşanan pek çok olay sonrasında ilaç geliştirme aşamaları uluslararası kabul edilen kurallar ile belirlenmiştir. Bu kurallara göre araştırılan maddenin etkisinin insanlardan önce hastalık modeli oluşturulmuş deney hayvanlarında araştırılması gerekmektedir. Bu amaçla geliştirilmiş pek çok hastalık modeli oluşturulmuştur. Deney hayvanlarında oluşturulan bu hastalık modellerinde geliştirilen pek çok ilaç günümüzde başarı ile insanların tedavisinde kullanılmaktadır.
Anahtar kelimeler: Farmakoloji; deney hayvanı; hastalık modelleri; ilaç; deney tasarımı.

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INTRODUCTION

The use of experimental animals in pharmacological scientific research has an important place among all pharmacological research. Especially in the last century, experimental animal research has played an important role in the development and introduction of modern medicines. In pharmacological scientific research, the design and planning of animal experiments are the most important steps for the success of the study. Each stage should be planned carefully, and waste of animals, time, and resources should not be allowed. The aim of this review was to give brief preliminary information on some issues that should be taken into consideration when using animal experiments in pharmacological scientific research and disease models in animals. Although some of the most used experimental animal models in the literature were included in this study, not all of them were mentioned.

ANIMALS IN PHARMACOLOGICAL RESEARCH

The use of experimental animals played an important role in the development of modern medicine. The reason for this is that although the substances whose effects are investigated on humans may differ in type, observing their effects in another living organism and physiological system produces more realistic results when applied to humans. That's why in vivo research has maintained its value for years. Studies with the first known experimental animal date back to 400 BC. Nobel Prize in Physiology between 1901 and 2020, or 186 scientists who won the Medicine (NPPM) award used experimental animals in their projects. 23 of these projects are directly related to pharmacology (1).

ANIMAL SPECIES USED IN SCIENTIFIC RESEARCH

Looking at the research, the preferred animal species in pharmacology research are mice, rats, rabbits, and guinea pigs. It is seen that there are pigs, pigs, dogs, and monkeys. Among these species, mice, rats, and rabbits are the most preferred. The reason for this is that these can be counted as their low body weight, their similarity to human anatomy (presence of similar organs), and their easy intervention. The type of animal to be used varies depending on the research goal. Even fish, pigeons, or helminths can be used (1). If surgery is to be performed in the research model, larger animals may be preferred. Although animal preferences vary depending on the disease model to be studied, many reasons should be taken into consideration, such as the amount and cost of the drugs to be administered, laboratory infrastructure, experience of the research, and compliance with the legislation. When we look at the percentage distribution of mammal species used in Nobel Prize-winning research, it is seen that the rodent species (mouse, rat) are the most common (42%), dogs (14%), rabbits (13%), and then other animal species (1).

PLANNING ANIMAL EXPERIMENTS IN PHARMACOLOGICAL RESEARCH

First of all, the problem should be determined through broad and comprehensive literature research. Afterward, a hypothesis regarding the problem should be put forward. The thing to consider in literature research is whether there has been a satisfactory publication on the subject you are researching. Trying to find an answer before is nothing but a waste of time and resources, except in some cases. After determining our hypothesis, it is necessary to determine the most accurate experimental method to prove it. This method may not always be an experimental animal research method in pharmacological research (Figure 1). If the best way to prove our hypothesis is through experimental animal research, we need to choose the most appropriate animal breed and disease model. When determining this choice, many issues such as our project budget, accessibility, the cost of the substance we will use for treatment, laboratory infrastructure, having a laboratory animal certificate, and the ability to apply the model should be considered. The 3R rule can help in this regard. The first R of the “3R” rule, one of today's bioethics rules, is “reduction”. This principle aims to keep the number of animals used in experiments as low as possible. Second R; “refinement” means to foresee and ensure the welfare and comfort of the animal. This principle aims to ensure that the living conditions of the animal are comfortable during the process, from the time it is selected for the experiment until its death. Third R: It is the principle of “replacement”. The purpose of this principle is that if the same results can be achieved with other experimental models other than experimental animals, they should be chosen first in the research. Today, “responsibility” has also been added to these principles. This principle can be summarized as conducting research by knowing the value of the experimental animal and complying with ethical rules (2). In light of these principles, research should be carried out by determining the minimum number of animals possible. Groups must be determined for the number of animals to be used. Statistical methods can be used for this. Reducing the number of animals in groups leads to less clarity as to whether the effect we are investigating is occurring. Groups with fewer than four animals each are considered non-consensual. If the therapeutic effect of a substance in a disease model is being investigated, it is generally recommended to use at least three different doses. This gives an idea about whether the drug-related effect is dose-dependent or not. Studies using a single dose are studies with less scientific competence. There must be control groups in studies. The group to which you applied the substance or method should be compared with groups to which no substance or method was applied. The purpose of this is to reveal whether the substance or method you are researching is different from normal or the effects of the research. If the substance or method you are researching does not reveal a significant difference compared to the control groups, it is considered to have no effect (3).

There are multiple types of control groups. Placebo control: In this group, animals are given a treatment that

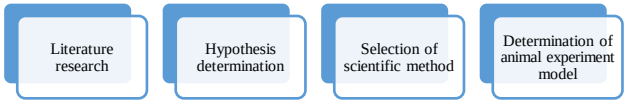

Figure 1. Experimental design algorithm

you think will not have any effect on the same treatment you give. For example, as a control of a drug administered to the gastrointestinal system via gavage, a 0.9% NaCl solution is given in the same way and the same amount. Here, it is investigated whether your medicine has any effect. Positive control: In this control group, a drug or substance that is currently used in the treatment of your disease model is administered to the control group. Here, it is investigated whether the effect of your medicine has a significant effect compared to the known treatment drug currently in use. Vehicle control: This control group, is aimed at investigating whether the substance used to make your medicine into a solution to administer it to animals has any effect on the disease model you are using and on the animals. Sham control: Here, if a serious surgical procedure is performed on the experimental animal during the formation of the disease model or sample collection (for example, abdominal operation and cecal ligation, carotid artery cannulation, etc.), it is aimed to reveal the effects of this operation on the experimental animal. The purpose of creating all these control groups is to reveal the pure and real effect of the treatment you apply (3).

To eliminate the gender effect in research, animals of the same sex are generally used in all groups. If your research includes hormonal effects that need to be avoided, male animals may be preferred. They also have to be the same genus and the same species. For example, while Wistar Albino male rats were used in one group of the same study, mice or rabbits could not be used in another group, and Spraque rats are not preferred in Dawley-type rats. Apart from this, animals of the same species and breed are distributed to groups randomly. Grouping animals with certain characteristics in a group creates a bias in the results of the experiment. Before starting your research, approval must be obtained from the Local Ethics Committee for Experimental Animals.

Information such as the purpose of your study, the experimental outline, treatments to be used, anesthetic drugs if used, and how euthanasia will be performed should be explained. The experiments must be carried out by a researcher who has a certificate for performing laboratory experiments on animals. If the researcher has no previous experience with the disease model to be used or has no experience with the substance to be used, pilot studies may need to be conducted before experiments. These studies play an important role in completing experiments in a shorter time and with less animal and material loss. These studies may take months (3).

Finally, when all permissions are obtained and the disease model is seen to be realized, the main experiments can be carried out. Experiments must be carried out meticulously, without any room for doubt, and every moment is recorded. The data obtained as a result of the experiment (such as EEG data, blood pressure data, hormone analysis data, genetic evaluation data, and pathological examination data) are recorded accurately and evaluated with statistical methods, and the results are obtained.

WAYS OF DRUG ADMINISTRATION

For the selection of drug administration methods, the disease model you will use in experimental animals and the type of experimental animal you will use are important. If the disease model you are planning is a systemic disease (diabetes, shock, etc.), the treatment you will apply must also be systemic. For example, local treatment methods come to the fore in models such as skin lesions or wound healing. Systemic drug administration routes are classified as enteral and parenteral (4). The enteral route can be summarized as the route used through the gastrointestinal system. Medicines used orally fall into this group. This group is defined as the most used and safest way to treat people today. In enteral drug use, the drugs can be mixed into the food and water of the animals, or the drug can be given directly into the animal's stomach by reaching the animal's stomach with various apparatus through the method we call gavage. The most important difference between these methods is that although it is natural to take the drug with food or drink, it is not possible to determine the exact amount of the drug administered. However, drugs given by gavage are given in a certain dose, and since it is ensured that it reaches the animal's stomach, there is no loss of drug dose. The gavage method is preferred, especially for drugs that will be used in low doses or have high costs. Gavage is the administration of medication through a syringe by placing a metal or plastic device orally into the animal's stomach. There are various sizes of gavage apparatus, depending on the breed and size of the animal. It is important to choose the appropriate apparatus according to the characteristics of the animal to avoid complications during application. Although the enteral route is mostly chosen for systemic treatments, it can also be used for local treatments in some disease (gastric ulcers, inflammatory bowel diseases, etc.) models (4). The parenteral route is the name given to drug administration routes other than enteral. Today, it is generally used for intravenous, intramuscular, or subcutaneous drug administration. These methods include percutaneous (on the skin) drug application, which is a local application method, as well as intraperitoneal (between the abdominal membranes) application, which is a systemic treatment application method that is more frequently used in experimental animals than in humans. In parenteral applications, drugs are generally used in specific and small doses via syringe. In parenteral administration, the duration of drug action is much shorter than in enteral administration. In intravenous administration, the effect of the drug can be seen within seconds. This period may take up to 1 hour for enteral applications (4).

EXPERIMENTAL ANIMAL DISEASE MODELS

The role of experimental animals has always been great in the development of drugs and treatments. In experimental animal research, to find the right treatments, the correct disease model must be created exactly in the experimental animal. Incomplete and inadequately created animal models cause both animal loss and research failure. For this reason, creating a disease model in experimental animals is the most important step of the research. This process can be long and challenging. The meticulousness of the researcher in creating a disease model will facilitate the success of the research. Especially in the last century, disease models created in experimental animals have developed considerably. Nowadays, an experimental animal model for almost every disease can be found (5).

Models of Central Nervous System Diseases

Models of common brain and spinal cord-related diseases as well as models of psychiatric diseases will be discussed in this section.

Epilepsy Models

Epilepsy models, which are disease models characterized by seizures, also known as "epilepsy" disease among the public, will be discussed. They are involuntary events that occur in the body because of pathologies experienced in the discharge of neurons. Symptoms such as convulsions and freezing are observed (6). Epilepsy models used in experimental animals were mentioned in Table 1.

Animal Models in Neurodegenerative Diseases

That may be seen as a result of degenerative changes in brain functions or that may progress with a decrease in the movement system or weakness in the muscles, such as amyotrophic lateral sclerosis (ALS) (11). Neurodegenerative disease models were mentioned in Table 2.

Table 1. Epilepsy models used in experimental animals

Method	Application Path	Mechanism of Effect
Focal penicillin model (7)	Systemic and application to the cortex surface in the brain	Stimulating effect of penicillin
Pentylenetetrazol, Bikukulin, Picrotoxin, Strychnine (8)	Systemic application	Glycine and Gaba receptor antagonism
Kainic acid (7)	Systemic application	Glutamate-like effect
Lithium-Pilocarpine (7)	Systemic application	Cholinergic-parasympathomimetic
Cobalt-Homocysteine (9)	Cobalt is placed in the brain	stimulating effect
Tetanus toxin (10)	Applied to certain parts of the brain	Glycine and Gaba release

Table 2. Neurodegenerative disease models used in experimental animals

Illness	Method
Alzheimer's (12)	Transgenic mice- Tg2576, PS1/APP, PDAPP
Alzheimer's (13)	Immune response model created by Aβ antibodies
Parkinson's (14)	6-hydroxydopamine, 1-methyl 4-phenyl, 1,2,3,6-tetrahydropyridine
Amyotrophic Lateral Sclerosis, ALS (15)	Transgenic mice- SOD1, TDP43, C21orf72

Mental Illnesses Models

These animal models are mostly used in the development of diseases such as depression and schizophrenia, which are considered within the scope of mental diseases.

Depression Patterns

This disease is characterized by symptoms such as unhappiness, reluctance, an inability to enjoy life and pessimism. Today, the diagnosis of this disease is made through a personal interview or examination. Since verbal communication is not possible in animals, the diagnosis is made based on findings such as a decrease in the daily movements of animals, a decrease in eating and drinking, and a decrease in communication with other animals. Models of this disease can be created with medication, behavioral tests, and genetic animal models (5).

Depression models used in experimental animals;

- **Drug Models**
  - Reserpine model (16)
  - Yohimbine toxicity model (17)
  - Apomorphine model (17)
  - Glucocorticoid/ Corticosterone model (18)
- **Behavioral Tests**
  - Forced buoyancy test (17)
  - Open field test (19)
  - Tail hanging test (16)
- **Genetic Models**
  - Finder's responsive array (18)
  - Holtzman albino strain (20)
  - Wistar-Kyoto type (21)
  - Transgenic models (18)

Schizophrenia Models

Schizophrenia is a chronic mental illness that distorts a person's perception of reality and causes delusions such as seeing or hearing things that do not actually exist. Experimental animals have an important place in research on this disease, for which no definitive treatment has yet been found.

Schizophrenia models used in experimental animals;

- **Pharmacological Models**
  - Amphetamine, Apomorphine-Dopaminergic agent application (22)
  - Phencyclidine, Ketamine- NMDA receptor antagonists (23)
- **Genetic Models**
  - DISC1 Deletion (24)
  - 22q11.2 Deletion (25)

Addiction Models

Addiction is among the most important health problems today. Experimental animals are used successfully in studies of drug-stimulant addiction as well as cigarette and alcohol addiction.

Addiction models used in experimental animals;

- **Patterns of Excessive Substance Use** (26)
  - Cocaine, Heroin
  - Fentanyl-Morphine
  - Nicotine-Cigarette
- **Animal Deprivation Models** (5,27)
  - Discontinuation of the addictive substance after becoming addicted
  - Antagonist drug administration
- **DSM-Based Animal Models** (28)

Models of Cardiovascular System Diseases

Hypertension Models

As is known, hypertension is a common disease among cardiovascular diseases. A lot of research is being done on the treatment of this disease, which progresses with an increase in blood pressure. Experimental animal models have an important place in these studies.

Hypertension models used in experimental animals;

- **Endocrine-Based Model**
  - Deoxycorticosterone acetate (DOCA) (29)
- **Diet-Induced Model**
  - High salt diet (30)
  - Obesity (31)
- **Model of Neurogenic Origin**
  - Hypothalamus and Rostral Ventero Lateral Medulla stimulation (32)
- **Genetic Model**
  - Spontaneously hypertensive rats - Inbred rat strain (33)

Myocardial Infarction Models

Today, the disease group that causes the most deaths in the world is still coronary artery disease. Despite the development of technology, the pharmaceutical industry, and the development of many new invasive treatments, it continues to have a high mortality rate. As a result, both acute myocardial infarction and the development of coronary artery stenosis are subject to extensive research. Rats are frequently used in these studies because the anatomy of the heart is very similar to the anatomy of the human heart. Among these models, some models require surgical skills.

Myocardial infarction models used in experimental animals;

- **Chemical Agent Application Model**
  - Isoprenaline: Synthetic sympathomimetic catecholamine (34)
- **Coronary Artery Ligation Model** (35)
- **Coronary Artery Embolization Model** (36)
  - Sponge foam, polystyrene microspheres, alcohol

Models of Respiratory System Diseases

Chronic obstructive pulmonary disease (COPD), which is common after smoking, and asthma, which occurs due to allergenic factors, are diseases that threaten a significant part of society. There is still no medicine or treatment that completely cures these diseases. However, many treatments have been developed to enable people with these diseases to continue their normal life comfort. Inhaled drug treatments, especially those developed in the last century, have both treated attacks and reduced the frequency of attacks.

COPD models used in experimental animals;

- Cigarette Smoke Model: Mouse, rat, Guinea Pigs, dog, monkey (5,37)
- Lipopolysaccharide Model: In aerosol form, in mice and rats (38)
- Elastase Model: Intranasal and tracheal administration in mice and rats (39)

Models of Gastrointestinal System Diseases

Gastrointestinal system diseases are among the most common diseases. These diseases, most of these, have definite treatments to be found despite still being treated and researched. A lot of illness has. These studies between inflammatory intestinal diseases and stomach ulcers are being conducted first.

Colitis and ulcer models used in experimental animals;

- **Colitis Models**
  - Dextran Sulfate Sodium (DSS) Model (40)
  - Trinitrobenzene Sulfonic Acid (TNBS) Model (41)
- **Ulcer Models**
  - Indomethacin (42)

Chronic Disease Models

Chronic diseases are diseases that last throughout people's lives. Since there is no definitive treatment for these diseases, the aim is to ensure the patient's life comfort as much as possible. The most prominent of these diseases is diabetes. Since much research has been done on diabetes, many animal models have emerged.

Diabetes models used in experimental animals;

- **Type 1 Diabetes Models**
  - Models Created with Chemical Substances - Alloxan (43)
  - Streptozocin - Antibiotic (43)
  - Non-obese diabetic (NOD) mouse (44)
  - Encephalomyelitis virus variant M (45)
- **Type 2 Diabetes Models**
  - Db/Db Diabetic Mouse (46)
  - Alloxan and Streptozocin (43)
  - Partial pancreatectomy (47)
- **Diet** (48)

Cancer Models

Today, most of the study was made on illness, none no doubt it is cancer. of this disease fatal to be, currently used of treatments side of the effects A lot and fatal possible new treatment find hoping made your studies to increase from where has happened. Most cancer types are caused by experimental animals. The models are also quite large.

Cancer models used in experimental animals;

- **Xenograft Models**
  - Transferring cancer tissue from a human to an animal (5)
  - With blood transfusion (5)
- **Syngenic Models**
  - Among creatures of the same species and genetics (5)
- **Transgenic Models**
  - Genetic models in which spontaneous neoplastic growth is stimulated (5)
- **Models Created with Carcinogenic Agents**
- **Leukemia Models**
  - High doses of gamma radiation or X-ray (49)
- **Lung Cancer Models**
  - Xenograph (50)
- **Thyroid Cancer Models**
  - Immunodeficiency syndrome mouse models with xenograft (51)
- **Breast Cancer Models**
  - N-Methyl-N- Nitrosourea injection in rats (52)
  - Transgenic model- Mouse mammary tumor virus (MMTV) (53)
- **Gastrointestinal System Cancer Models**
  - Hepatocellular Carcinoma Model: Diethylnitrosamine (DEN) (54)
  - Gastric Cancer Model: N-Methyl-N- Nitro -N-Nitrosoguanidine (MNNG) (55)
  - Colorectal Cancer Model: Azoxymethane (AOM) /1,2-Dimethylhydrazine (DMH) (56)

Other Models

Frequently used above and on a lot more study-made experimental illness from the models has been mentioned. In addition to these models, infectious diseases, eye, ear, and nose throat, dermatology, urology, nephrology, and orthopedics have successfully experimented with bestial illness models.

ANIMAL EXPERIMENTS IN TOXICOLOGY RESEARCH

One of the most important stages of drug development is toxicological testing. Toxicology tests are necessary to determine the toxic effects of new drugs and, therefore, the safety of drugs. These toxic effects are investigated first in vitro in cell cultures and then in vivo in experimental animals. Toxicology tests performed are classified according to the duration of drug exposure (57):

- Acute toxicity tests
- Subacute toxicity tests
- Subchronic toxicity tests
- Chronic toxicity tests
- Special toxicity tests

As a result of these tests, important parameters of the toxicity of a drug or substance are the LD50 value, which is the dose that kills 50% of the animals in the experimental group when given a single dose, and the concentration LC50 value of the drug or substance that kills 50% of the animals in the experimental group after exposure for a certain time. These results are the basic values for finding the therapeutic dose of the drug. LD50 and LC50 values of many substances have been found for both animals and humans (57).

SAMPLE COLLECTION METHODS

An important step in the design of animal experiments in pharmacological research is how to obtain the results of the substance for which you create a disease model and apply it for treatment. For this purpose, except for observational studies, samples must be collected from the experimental animal. These samples must be collected in a way that suits our purpose, does not affect the experimental results, and does not harm the welfare of the animal. When planning the research, it should be determined in advance which sample, in what quantity, at what time, and how you will collect it, and how and in what environment these samples will be stored until analysis. These preparations must be completed at the time of sample collection. For example, if a blood sample is taken, will it be serum? plasma? should be determined. Accordingly, it should be determined which blood collection tube should be used. If histopathological examination is to be performed, tissue samples should be placed in formalin solution without delay. Tissues that should not be exposed to chemicals should be immediately placed in a -800 C freezer or liquid nitrogen. If blood samples are to be stored for a short time, they can be stored in a -200 C deep freezer, but if they are to be stored for a long time, they can be stored in a -800 C deep freezer. In studies conducted with brain tissues, a hot water bath, and tissue oxygenation are required. There are also special sample collection methods. One of these is the microdialysis method. It is based on the principle of placing a specially prepared microdialysis apparatus in the brain region of the experimental animal to be examined and removing the neurotransmitters or substances in that region by the dialysis method. It is a very sensitive method and provides important data in brain research (58).

Main sampling types used in experimental animals;

- Taking a blood sample
- Collecting tissue samples
- Stool and urine collection
- Microdialysis
- Viewing and recording
- Bile
- Lymph fluid
- Cerebrospinal fluid
- Peritoneal ascitic fluid

SAMPLE ANALYSIS METHODS

The last and most important step of pharmacological research is the analysis of the data or samples you collect. At this stage, the data and samples obtained from the experimental animals should be analyzed. How this analysis will be carried out should be determined meticulously during the planning phase of your research. Because choosing the wrong analysis method can lead to your research being wasted. The analysis method may vary depending on the type of sample you collect. If you are going to measure biochemical parameters (AST, ALT, CRP, ALP, glucose, urea, etc.), you can get results with a blood sample on devices that investigate these parameters. If you are going to measure a substance that may be present in small amounts in the body, you can use analytical devices called HPLC, LC-MS/MS, or GC-MS to measure very small amounts (nmol, pmol). You can choose sensitive devices that can even analyze substances (e.g., etc.). If you want to examine the effects at the cellular level, you can take relevant tissue samples from the experimental animal and have a histopathological examination done. While some of these analysis methods are practical tests that can be performed by the researcher, some of them are methods that require expensive devices or experts in the field. For this reason, when planning at the very beginning of the research, it is necessary to take into account the budget of the research, the researcher's experience in the analysis, and the possibilities of accessing the analysis.

Some of the main analysis methods used in experimental animals;

- Histopathological Analyzes
- Analyzes with Analytical Devices- HPLC, LC-MS/MS, GC-MS
- Immunoassay Methods
- Imaging analytics

DISCUSSION

The use of experimental animals is of vital importance in pharmacological research. A drug planned to be used in humans must be tested in living systems like humans before it is offered to humans. Because drugs, which are chemical substances, can exhibit a very different behavior in a living system in vivo than in an in vitro environment, There are many reasons for this. Living systems are like machines with many gears intertwined. As a result of the malfunction of one of these wheels, the life of a living

creature may end or it may be seriously harmed. For this reason, whether a substance that does not belong to the body will harm these gears can only be tested in systems with similar gears. Today, despite all this technological progress, nothing like the human living system has been created. For this reason, the closest system we currently have is the system of experimental animals.

Considering all these stages, the design of experimental animals in pharmacological research must be prepared carefully and meticulously. Ultimately, the aim is to obtain real and accurate results.

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