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A REVIEW ON ANIMAL STUDIES IN DRUG DEVELOPMENT-TUBERCULOSIS

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ABSTRACT :

Experimental Pharmacology deals with the effect of various pharmacological agents studied on different animal species. The domain "Experimental Pharmacology" comprises with 3 modules with different matters. Module one deals with etiology and pathophysiology of Tuberculosis. The second Module illustrated with diagnosis and treatment of Tuberculosis. The third module includes preclinical studies and regulations. The fourth Module describes the various screening methods for the study of antitubercular drugs.

INTRODUCTION :

Tuberculosis is an infectious disease that can cause infection in your lungs or other tissues. It commonly affects your lungs, but it can also affect other organs like your spine, brain or kidneys. The word “tuberculosis” comes from a Latin word for "nodule" or something that sticks out. Tuberculosis is also known as TB. About 10 million people became ill with TB throughout the world, and about 1.5 million people died from the disease in 2020. Tuberculosis (TB) is a contagious infection that usually attacks your lungs. It can also spread to other parts of your body, like your brain and spine. A type of bacteria called Mycobacterium tuberculosis causes it. People with HIV/AIDS and others with weakened immune systems are at higher risk of getting tuberculosis because their bodies have a harder time fighting the bacteria.

“Tuberculosis is the infectious disease caused by several species of mycobacterium”. It spreads very rapidly from one person to the other directly or indirectly. According to WHO; tuberculosis is caused by bacteria (mycobacterium tuberculosis) that most often affect the lungs. Tuberculosis is curable and preventable.

TYPES :

Tuberculosis can be classified into different clinic-pathological types depending on various factors based on:

- The sequence of events following the first exposure
- Location

Three types of tuberculosis arise when the disease is classified according to the sequence of events following the first exposure, such as:

- Primary tuberculosis - Occurs in persons exposed to Mycobacterium tuberculosis for the first time.
- Progressive primary tuberculosis - Arises when there is impaired immunity. It is most commonly seen in infants, adolescents and the elderly.
- Post-primary tuberculosis - Generally seen in adults due to endogenous reactivation or exogenous reinfection in a previously sensitised (infected and treated) patient who has retained some degree of acquired immunity.

Based on the location of the infection, two types of tuberculosis are seen, such as:

- Pulmonary tuberculosis - tuberculosis is seen in the lungs
- Extrapulmonary tuberculosis - Occurs in organ systems other than the lungs. The most common sites are lymph nodes, pleura, bone and joints, urogenital tract, and meninges.

SYMPTOMS :

A cough that lasts more than 3 weeks

- Chest pain
- Coughing up blood
- Feeling tired all the time
- Night sweats
- Chills
- Fever
- Loss of appetite

- Weight loss
- When TB is outside the lungs, you may have these same symptoms along with pain near the area that's infected.

RISK FACTOR

- A friend, co-worker, or family member has active TB.
- You live in or have travelled to an area where TB is common, like Russia, Africa, Eastern Europe, Asia, Latin America, and the Caribbean.
- Your part of a group in which TB is more likely to spread, or you work or live with someone who is.
- You work or live in a hospital or nursing home.
- You're a health care worker for patients at high risk of TB.
- You're a smoker.
- Low body weight and poor nutrition.
- Immunocompromised patients.

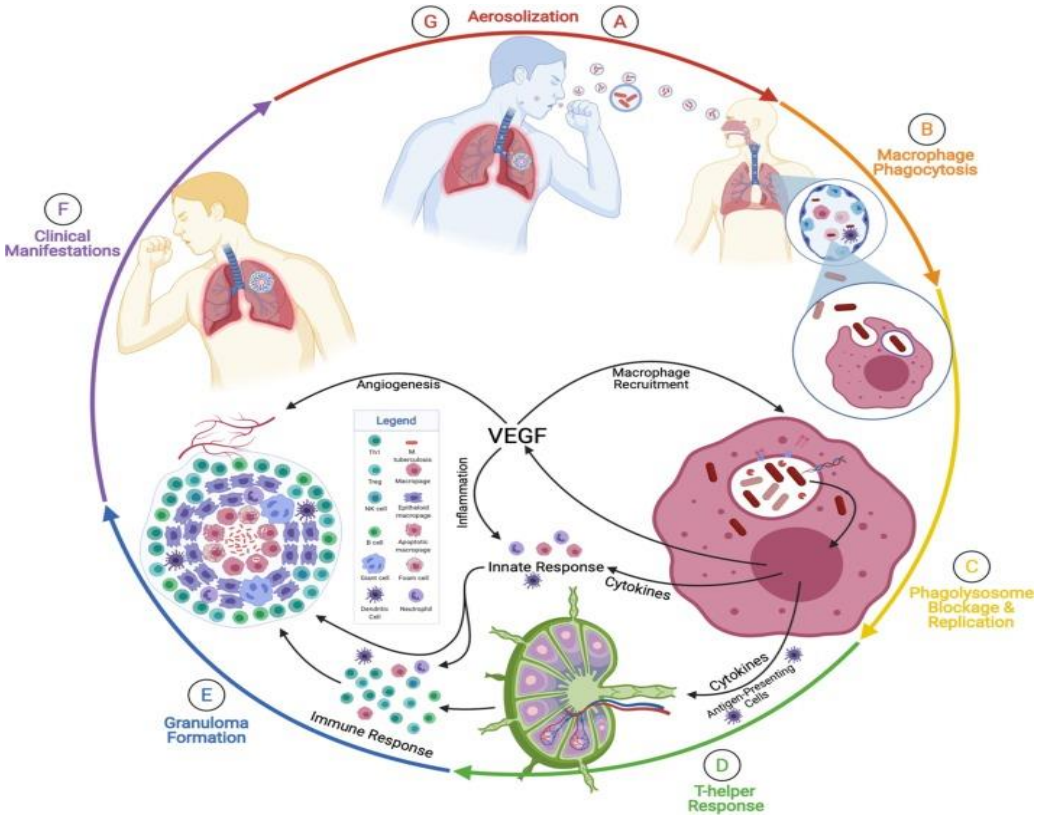
TRANSMISSION

TB germs can get into the air when a person with active TB disease of the lungs or throat coughs, speaks, or sings. These germs can stay in the air for several hours, depending on the environment. TB germs are more likely to spread in indoor areas or other places with poor air circulation (such as a closed vehicle) than in outdoor areas. People nearby may breathe in these germs and become infected.
TB germs are not spread by:

- Shaking someone's hand
- Sharing food or drink
- Touching bed linens or toilet seats
- Sharing toothbrushes
- Kissing

ETIOLOGY

TB is an infectious disease caused by bacteria of the Mycobacterium tuberculosis complex, of which M. tuberculosis is the most common and important agent causing human disease.
Similar disease occasionally results from the closely related mycobacteria, M. bovis, M. africanum, and M. microti.
In 1882, Robert Koch demonstrated that the tubercle bacillus was the true cause of TB, a discovery for which he received noble prize in 1905.
TB bacteria can live in the body without making you sick. This is called inactive TB, or latent TB infection. People with inactive TB are infected with TB germs, but they do not have active TB disease. They do not feel sick, do not have symptoms of TB disease, and cannot spread TB germs to others.



PATHOPHYSIOLOGY

The pathophysiology of Mycobacterium tuberculosis infections, known as tuberculosis, is a concert of interplay between pathogenic and physiological processes. M. tuberculosis has evolved to thrive by using the human immune system to gain access to the host and remain within the host for years.
These steps are aerosolization, macrophage phagocytosis, phagolysosome blockage and replication, T helper type 1 (TH1) response, granuloma formation, clinical manifestations, and transmission.
A) Aerosolization is the beginning and the end of the cycle of tuberculosis pathophysiology. Aerosolization occurs when a person with active tuberculosis forcefully expires through actions such as coughing.
B) A susceptible person who breathes in the aerosolized Mycobacterium tuberculosis and droplets small enough to reach the alveolar sacs (shown in the first magnification) will encounter macrophages, dendritic cells, and monocytes. The macrophages will phagocytose the bacteria (shown in the second magnification) and attempt to destroy the invader. Dendritic cells will migrate to lymph nodes to activate T-helper cells.
C) M. tuberculosis prevents the phagolysosome fusion, avoids destruction, begins replicating, and releases DNA, RNA, proteases, and lipids. Additionally, the macrophages will release cytokines and vascular endothelial growth factor (VEGF). The VEGF will trigger angiogenesis and increase vascularization to the lesion. The cytokines will initiate the innate response and recruit natural killer cells, dendritic cells, neutrophils, and macrophages in different forms.
D) The T-helper cell response will involve the migration of TH1, Tregs, and B cells primed in the germinal center. These cells will combine to form the granuloma
(E). The granuloma is a prison to wall off the bacteria from spreading systemically.
F) Later, or present, immune compromisation prevents the granuloma from containing the bacteria. The bacteria will spread and multiply in multiple clinical manifestations.
G) During this phase, the bacteria can be aerosolized by the original susceptible, now infected, host, and begin the cycle anew.

DIAGNOSIS

Different tests are used to diagnose TB, but a healthcare provider will usually start by checking for swollen lymph nodes and listening to someone's breathing with a stethoscope. Next, they'll likely do some additional testing to determine whether someone has active or latent TB.

Mantoux tuberculin skin test (TST)

TST is performed by injecting a small amount of tuberculin into the skin of the forearm. The skin will be monitored for a reaction 48 to 72 hours after the injection. A positive skin test indicates that TB bacteria is present, and additional tests are needed to determine if it's active or latent.

Blood tests

These tests, also called interferon-gamma release assays (IGRAs), measure the response when TB proteins are mixed with a small amount of your blood. Blood tests can help to confirm or rule out active or latent TB. The tests measure your immune system's reaction to TB bacteria. There are two blood tests approved by the Food and Drug Administration for TB:
T-SPOT TB test (T-Spot)
QuantiFERON-TB Gold In-Tube test (QFT-GIT).

If your skin test results are positive, you most likely have TB bacteria. But you could also get a false positive. If you've gotten a tuberculosis vaccine called Bacillus Calmette-Guerin (BCG), the test could say you have TB when you really don't. You can also get a false negative, saying that you don't have TB when you really do, if your infection is very new.

Imaging tests

Following a positive skin test, a healthcare provider may order a chest X-ray or CT scan. These tests produce images that may show changes in the lungs caused by active TB.

Sputum tests

Sputum is the mucus that comes up when you cough. Healthcare providers sometimes collect sputum samples and test them for different strains of TB bacteria, including antibiotic-resistant types. The results of sputum tests are helpful in choosing the best course of treatment.

TREATMENT :

NON-PHARMACOLOGICAL TREATMENT

- Adequate nutrition
- Medico social awareness
- Less crowded living conditions
- Avoid dust
- Avoid smoking

PHARMACOLOGICAL TREATMENT

TB treatment is effective. Worldwide, nearly 90% of cases of TB and 48% of cases of drug-resistant TB are cured. However, treatment is not quick or easy. The length of treatment and side effects from the drugs used pose huge problems for TB patients and for global efforts to tackle the disease. Find out more under Global TB challenges.

TB treatment lasts at least six months. Treatment for TB is usually a mixture of four antibiotics:

First Line:

- Rifampin10mg/kg-
- Isoniazid-5mg/kg
- Pyrazinamide-30-40mg/kg
- Ethambutol-15-25mg/kg

Second Line:

- Kanamycin (discontinued use in the USA)
- Streptomycin
- Capreomycin
- Amikacin

Isoniazid, Rifampicin and Pyrazinamide can come in the same tablet, called Rifater. After two months of being on this treatment, patients may then be moved on to a course of two antibiotics for four months: Rifampicin and Isoniazid. These can come in the same tablet, called Rifinah.

Patients may begin to feel better within two weeks of beginning treatment, and people with pulmonary TB normally become non-infectious during this time. However, it's vital that patients complete their treatment, so that the TB bacteria are completely killed off in the body. This prevents symptoms from returning and the risk of bacteria becoming drug resistant.

Treating drug-resistant TB

Drug-resistant TB requires a longer course of treatment, with different combinations of drugs that can have more side effects. A patient will be tested to find out the exact course of treatment that should work for them.

Bedaquiline
Delamanid
Linezolid
Pretomanid

Treating latent TB

Most cases of latent TB are not considered for treatment, as 90% of people with latent TB do not go on to become ill with active TB. Treatment is recommended for people whose immune systems are weaker as they are more likely to go on to develop an active infection. This includes children and people living with HIV. The dormant bacteria present in cases of latent TB can be cleared completely using some of the same drugs used to treat active TB.

Combinational Therapy

During the 2-mo intensive phase, patients should be administered a combined regimen including ethambutol, isoniazid, pyrazinamide, and rifampicin. Only isoniazid and rifampicin are prescribed during the 4-mo continuation phase. Current treatment regimens for drug-susceptible tuberculosis typically achieve cure rates of 85% for new cases of tuberculosis and can achieve cure rates as high as 98%; however, even these first-line regimens require the use of four antimicrobial drugs over the course of 6 months .The necessity for multiple drugs in treating tuberculosis is driven by several factors concerning the causative organism M. tuberculosis, including but not limited to the general recalcitrance of M. tuberculosis with respect to treatment due to its peculiar cellular structure and metabolism; the propensity of M. tuberculosis to persist in the face of drug treatment and/or attack by the host immune system; and the tendency of M. tuberculosis to develop resistance to drug therapy (4–6). As a result, treatment of tuberculosis has required the combination of several antimicrobial drugs since the first applications of drug therapy to the disease.

Eg; **R**(150mg) +**H**(75mg) +**Z**(400mg) +**E**(275mg)
R(150mg) +**H**(75mg) +**Z**(400mg)

Directly Observed Treatment (DOT)

TB treatment takes at least six months, patients need to take many tablets each day and side effects are common. This can be very difficult for people to manage, but it's crucial that they take their treatment as prescribed and complete the course, to ensure they are completely cured and prevent them developing drug-resistant treatment.

Directly Observed Treatment, or DOT for short, is a highly successful way of supporting people to complete their treatment. DOT involves TB nurses, outreach volunteers or trained volunteers meeting regularly with patients to watch them take their medication. This may take place at the patient's home, in a clinic or pharmacy, or even a local shop. DOT ensures that the right medication is taken in the right doses, at the right time, for as long as it's required. In the UK, DOT is recommended for people who have difficulty keeping to a course of treatment – perhaps through lifestyle factors such as homelessness – and for people affected by MDR-TB.

BCG Vaccine

The Bacillus Calmette–Guérin (BCG) vaccine is a vaccine primarily used against tuberculosis (TB). It is named after its inventors Albert Calmette and Camille Guérin. In countries where tuberculosis or leprosy is common, one dose is recommended in healthy babies as soon after birth as possible. In areas where tuberculosis is not common, only children at high risk are typically immunized. Among children, it prevents about 20% from getting infected and among those who do get infected, it protects half from developing disease. The vaccine is given by injection into the skin.
Serious side effects are rare. Often, redness, swelling, and mild pain occur at the site of injection. The vaccine was originally developed from Mycobacterium bovis, which is commonly found in cattle. While it has been weakened, it is still live.

PRECLINICAL STUDIES :

Preclinical studies using animals to study the potential of a therapeutic drug or strategy are important steps before translation to clinical trials. However, evidence has shown that poor quality in the design and conduct of these studies has not only impeded clinical translation but also led to significant waste of valuable research resources. It is clear that experimental biases are related to the poor quality seen with preclinical studies.
Preclinical research is generally divided into four phases – basic research, drug discovery, lead optimization, and IND-enabling studies. Identifying a safe, potent, and efficacious drug requires thorough preclinical testing, which evaluates aspects of pharmacodynamics, pharmacokinetics, and toxicology in in vitro and in vivo settings.
Each class of product may undergo different types of preclinical research. For instance, drugs may undergo pharmacodynamics (what the drug does to the body) (PD), pharmacokinetics (what the body does to the drug) (PK), ADME, and toxicology testing. Most preclinical studies must adhere to GLPs in ICH Guidelines to be acceptable for submission to regulatory agencies such as the Food & Drug Administration in the United States.
Typically, both in vitro and in vivo tests will be performed. Studies of drug toxicity include which organs are targeted by that drug, as well as if there are any long-term carcinogenic effects or toxic effects causing illness.

INSTITUTIONAL ANIMAL ETHICAL COMMITTEE :

Institutional Animal Ethics Committee (IAEC) has been constituted to oversee and evaluate all aspects of the institution's animal care and use program.
Function of IAEC:
IAEC should provide independent, competent and timely review of the ethics of a proposed study before the commencement of the same and regularly monitor the ongoing studies.
IAEC will review and approve all research proposals involving animal experiments with a view to assure quality maintenance and welfare of animals used in laboratory studies while conducting research.
Composition of IAEC:
Institutional Animal Ethics Committee shall include members as follows:

- ✓ A scientist from different biological discipline cum chairperson
- ✓ A scientist from different biological discipline
- ✓ A Biological Scientist
- ✓ One veterinarian involved in the care of animals
- ✓ A scientist in charge of animal House facility cum member secretary
- ✓ A scientist from outside the institute
- ✓ One non-scientific socially aware person
- ✓ One main nominee of CPCSEA
- ✓ One link nominee of CPCSEA

CCSEA GUIDELINES FOR LABORATORY ANIMAL FACILITY

Good Laboratory Practices (GLP) for animal facilities is intended to assure quality maintenance and safety of animals used in laboratory studies while conducting biomedical and behavioural research and testing of products.

GOAL

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioural research and testing with the basic objective of providing specifications that will enhance animal well-being, quality in the pursuit of advancement of biological knowledge tl1at is relevant to humans and animals.
CARE AND MAINTAINENCE OF LABORATORY

VETERINARY CARE

Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience 'in laboratory animal sciences and medicine. Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and

frequent communication should be adopted so that timely and accurate information on problems in animal health, behaviour, and well-being is conveyed to the attending veterinarian.

QUARANTINE, STABILIZATION AND SEPARATION

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. A minimum duration of quarantine for small lab animals is one week and larger animals is 6 weeks (cat, dog, monkey, etc.)
The length of time stabilization will depend on the type and duration of animal transportation, the species involved and the intended use of the animals. Physical separation of animals by species is recommended to prevent interspecies disease transmission.

ANIMAL PROCUREMENT AND TRANSPORT

The transport of animals from one place to another is very important and must be undertaken with care. The main considerations for transport of animals are, the mode of transport, the containers, the animal density in cages, food and water during transit, protection from transit infections, injuries and stress. The mode of transport of animals depends on the distance, seasonal and climatic conditions and the species of animals. Animals can be transported by road, rail or air taking into consideration of above factors. In any case the transport stress should be avoided and the containers should be of an appropriate size so as to enable these animals to have a comfortable, free movement and protection from possible injuries.

EXPERIMENTAL AREA

All experimental procedures in small animals should be carried out in a separate area away from the place were animals. are housed. For larger animal functional areas for aseptic surgery should include a separate surgical support area, a preparation area, the operating room or rooms, and an area for intensive care and supportive treatment of animals.

PHYSICAL FACILITIES :

(a) **Building materials** should be selected to facilitate efficient and hygienic operation of animal facilities.
(b) **Corridor(s)** should be wide enough to facilitate the movement of personnel as well as equipment and should be kept clean.
(c) **Utilities** such as water lines drain pipes, and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.
(d) **Animal room doors** should be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window.
(e) **exterior windows** are not recommended for small animal facilities.
(f) **floors** should be smooth, moisture proof, non-absorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants.
(g) **Drains** are not essential in all rooms used exclusively for housing rodents. Floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds.
(h) **Walls &ceilings** should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners.
(i) **Storage areas** Separate storage areas should be designed for feed, bedding, cages and materials not in use.
(j) **Facilities for sanitizing equipment and supplies** an area for sanitizing cages and ancillary equipment is essential with adequate water supply.

ANIMAL HUSBANDRY

- **CAGING OR HOUSING SYSTEM**

The caging or housing system is one of the most important elements in the physical and social environment of research animals. It should be designed carefully to facilitate animal wellbeing, meet research requirements, and minimize experimental variables.
The housing system should:

- Provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species
- Provide a comfortable environment
- Provide an escape proof enclosure that confines animal safety
- Provide easy access to food and water
- Provide adequate ventilation

- **BEDDING**

Bedding should be absorbent, free of toxic chemicals or other substances that could injure animals or personnel, and of a type not readily eaten by animals. Bedding should be used in amounts sufficient to keep animals dry between cage changes without coming into contact with watering tubes.

- **WATER**

Ordinarily animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their particular requirements. Periodic monitoring of microbial contamination in water is necessary. Watering devices, such as drinking tubes and automatic waterers if used should be examined routinely to ensure their proper operation.

❖ **SANITATION AND CLEANLINESS**

Sanitation is essential in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris, and harmful contamination. Cages should be sanitized before animals are placed in them. Deodorizers or chemical agents other than germicidal should not. be used to mask animal odours. Such products are not a substitute for good sanitation.

❖ **FOOD**

Animals should be fed palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise.

Feeders should allow easy access to food, while avoiding contamination by urine and feces. Food should be available in a mounts sufficient to ensure normal growth in immature animals and maintenance of normal body weight, reproduction, and lactation in adults. Food should contain adequate nutrition, including formulation and preparation; freedom from chemical and microbial contaminants; bioavailability of nutrients should be at part with the nutritional requirement of the animal. Laboratory animal diets should not be manufactured or stored in facilities used for farm feeds or any products containing additives such as rodenticides, insecticides, hormones, antibiotics, fumigants, or other potential toxicants. Areas in which diets are processed or stored should be kept clean and enclosed to prevent entry of insects or other animals.

❖ **WASTE DISPOSAL**

Wastes should be removed regularly and frequently. All waste should be collected and disposed in a safe and sanitary manner. The most preferred method of waste disposal is incineration. Waste cans containing animal tissues, carcasses, and hazardous wastes should be lined with leak - proof, disposable liner. Wastes must be stored before removal, the waste storage area should be separated from other storage facilities and free of flies, cockroaches, rodents, and other vermin. Cold storage might be necessary to prevent decomposition of biological wastes.' Hazardous wastes should be rendered safe by sterilization, contamination, or other appropriate means before they are removed from an animal facility for disposal.

❖ **PEST CONTROL**

Programs designed to prevent, control, or eliminate the presence of or infestations by pests are essential in an animal environment.

❖ **EMERGENCY, WEEKEND AND HOLIDAY CARE**

Animals should be cared for by qualified personnel every day, including weekends and holidays, to safeguards their well - being including emergency veterinary care. In the event of an emergency, institutional security personnel and fire or police officials should be able to reach people responsible for the animals.

❖ **RECORD KEEPING**

The Animal House should maintain following records:

- Animal House plans,
- Animal House staff record
- Health record of staff and animals.
- All SOPs relevant to the animals
- Breeding, stock, purchase and sales records
- Minutes of institute Animals Ethics Committee Meetings
- Records of experiments conducted with the number of animals used
- Death Record
- Clinical record of sick animals.
- Training record of staff involved in animal activities
- Water analysis report

❖ **STANDARD OPERATING PROCEDURES**

The Institute shall maintain SOPs describing procedures / methods adapted with regard to Animal Husbandry, maintenance, breeding, animal house microbial analysis and experimentation records.

A SOP should contain the following items:

- Name of the Author
- Title of the SOP
- Date of preparation
- Reference of previous SOP on the same subject and date
- Location and distribution of Sops with sign of each recipient Objectives
- Detailed information of the instruments used. in relation with animals with methodology
- The name of the manufacturer of the reagents and the methodology of the

analysis pertaining to animals

- Normal value of all parameters
- Hazard identification and risk assessment

❖ **ANASTHESIA AND EUTHANASIA**

The scientists should ensure that the procedures, which are considered painful, are conducted under appropriate anaesthesia as recommended for each species of animals. It must also be ensured that the anaesthesia is given for the full duration of experiment and at no stage the animal is conscious to perceive pain during the experiment.

In the event of a decision to sacrifice an animal on termination of an experiment or otherwise an approved method of euthanasia should be adopted and the investigator must ensure that the animal is clinically dead before it is sent for disposal.

Anaesthesia

Unless contrary to the achievement of the results of study, sedatives, analgesics and anaesthetics should be used to control pain or distress under experiment. Anaesthetic agents generally affect cardiovascular, respiratory and thermoregulatory mechanism in addition to central nervous system. Before using actual anaesthetics, the animals are prepared for anaesthesia by overnight fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardiopulmonary system and reduce salivary secretion. Atropine is most commonly used anti-cholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure.

Euthanasia

Euthanasia is resorted to events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons. The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. For accepting a euthanasia method as humane it should have an initial depressive action on, the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species of animal to be killed.

OECD Guidelines

Acute Oral Toxicity – Acute Toxic Class Method

- OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress or changing assessment practices. The original Guideline 423 was adopted in March 1996 as the second alternative to the conventional acute toxicity test, described in Test Guideline 401. Based on the recommendations of several expert meetings, revision was considered timely because:

i) international agreement has been reached on harmonised LD50 cut-off values for the classification of chemical substances, which differ from the cut-offs recommended in the 1996 version of the Guideline testing in one sex (usually females) is now considered sufficient.

- The acute toxic class method (1) set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods. The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment

SCREENING METHODS :

Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), is a life-threatening infectious disease, which was responsible for 1.3 million deaths globally. The treatment regimen for TB mainly comprises antibiotics such as rifampicin (RIF), isoniazid (INH), ethambutol, pyrazinamide, and aminoglycosides. Therapy with these antibiotics must be taken for 6 months to 2 years, which can result in the emergence of drug resistance and poor therapeutic outcomes. With the emergence of multidrug-resistant and extensively drug-resistant strains tackling the disease using currently available therapeutic regimens is a huge challenge. Therefore, there is a desperate need for novel, effective anti-TB drugs that can render the bacteria more susceptible to treatment.

Bacterial Strain and Culture Conditions

The Mtb H37Ra used in this study was obtained from the American Type Culture Collection (ATCC25177) and maintained in Difco Middlebrook 7H9 broth (Becton Dickinson), supplemented with 0.5% glycerol, 0.05% Tween 80, and 10% oleic acid albumin dextrose catalase (OADC, BD, United States) at 37°C. For bacterial culture and infection, all experiments were carried out in a biosafety level 2 laboratory following the appropriate biosafety standard operating procedures.

INVITRO METHODS:

- **Microplate Alamar Blue Assay**

Mycobacterium tuberculosis H37Ra was cultured to mid-log phase, with an optical density (OD) of 0.6 (~5 × 107 colony-forming units [CFU]/ml) at 600 nm. One hundred microliters (5 × 104 CFUs) of bacterial suspension were added to each well of 96-well microplates. Then, 10 μM of the compound was added to each well. DMSO was used as the negative control, and rifampicin (RIF) was used as the positive control. The plates were sealed and incubated at 37°C for 6 days. A 10% (v/v) solution of Alamar Blue was then added to each well. The anti-Mtb effect of each compound was determined based on the color change. The compound was considered to be inactive against Mtb if the color changed from blue to red.

RESULT :

The large-scale screening of the compounds in microplates showed that antibiotics including orbifloxacin, prulifloxacin, nadifloxacin, disulfiram, and mithramycin, and antimicrobial peptides exhibited significant anti-Mtb activity. Amongst the compounds tested, the fluoroquinolones (orbifloxacin, prulifloxacin, and nadifloxacin) and mithramycin were found to show the best anti-TB activity.

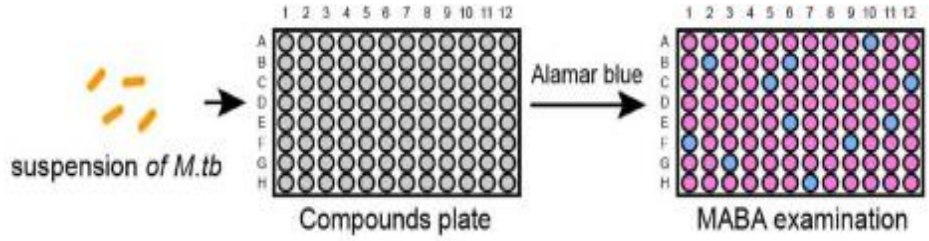


FIGURE 2

INVIVO METHODS

- **CYTOKINE ASSAY**

6 mice (female mice, 6−8 weeks old, 20 ± 2 g) were raised under specific pathogen-free conditions at the Laboratory. All animals were randomly divided into three groups of 6–8 mice in each. Mice were infected intravenously with 0.2 ml Mtb H37Ra suspension at a dose of 5 × 106 CFU/ml for 4 days. The infected mice were then orally administered orbifloxacin at a dose of 50 mg/kg per day. Isoniazid was used as a positive control at a dose of 12.5 mg/kg, and PBS was used as a negative control. The drugs were administered to the mice for 7 days post of infection. The mice were euthanized 18 days after infection. RNA was extracted from the lungs of mice, using the chloroform–isopropanol method. The lungs were used for histopathological and CFU analysis.

RESULT

The mice in the orbifloxacin and INH groups (isoniazid used as a drug positive control) showed a slight weight loss with a significant reduction in the number of CFUs relative to that in the PBS control. There was no significant difference between the groups treated with orbifloxacin and INH. Histopathological examination of lung tissues revealed that PBS-treated mice showed moderately higher infiltration of inflammatory cells, such as macrophages and lymphocytes, than mice treated with the drugs (Figure 3C). In PBS-treated mice, thickening of the alveolar wall, narrowing of the alveolar space, and the buildup of some red blood cells in the cavity were observed in the lungs. In contrast, the lesions were much milder and fewer macrophages were present in the alveolar cavity of the mice treated with orbifloxacin. We also analysed cytokine expression levels in the lungs of the mice. IL-6 and IL-1β expression were significantly lower in the orbifloxacin-treated group than in the PBS-treated group. There were no significant differences in the expression of TNF-α in any of the groups. The expression of IL-4, the representative cytokine of Th2 response showed slightly decrease in orbifloxacin treated mice (Figure 3E). IL-10 and IFN-β expression were significantly higher in the orbifloxacin-treated group than in the INH- or PBS-treated groups, suggesting that orbifloxacin can protect mice from a strong inflammatory response. Overall, our results show that orbifloxacin exhibits strong in vivo anti-Mtb activity and could be considered as a potential drug for the treatment of TB.

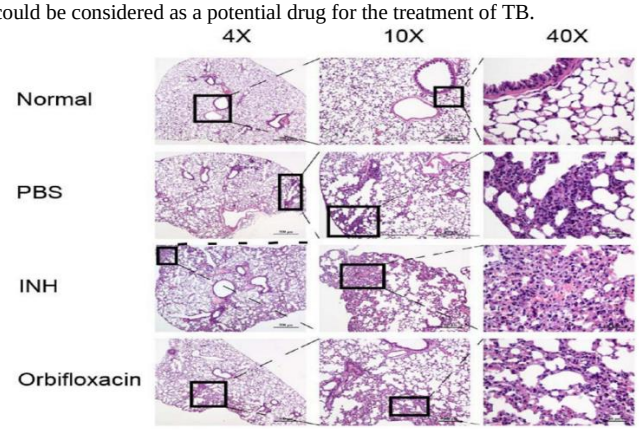


FIGURE 4

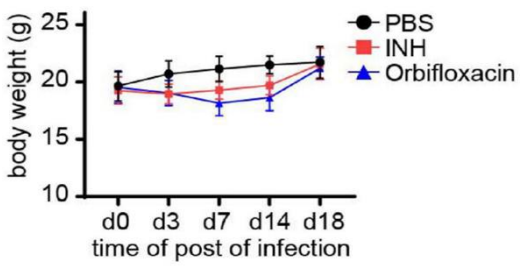


FIGURE 3

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