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A REVIEW ON PRECLINICAL PHARMACOLOGY AND TOXICOLOGY

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Abstract:

Preclinical pharmacology and toxicology are essential elements of the drug discovery and development process and are critical in enabling the translation of findings from the laboratory and the clinic. The drug discovery process is complex and involves numerous iterative steps designed to optimize the pharmacological and drug-like properties of a candidate molecule, a New Chemical Entity (NCE), and minimize the potential for side effects and toxicities. Key concepts addressed in this record include: compound identification; lead optimization; pharmaceutical profiling; the use of animal models to predict efficacy and safety and toxicological assessment as these relate to the regulatory requirements for Phase I trial initiation. Commentary is also provided on the current challenges associated with translational medicine as it applies to the effective evolution of candidate NCEs into viable clinical candidates.

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INTRODUCTION:

The GLP-1 receptor agonist exenatide is synthetic exendin-4, a peptide originally isolated from the salivary secretions of the Gila monster. Exenatide was developed as a first-in-class diabetes therapy, with immediate- and extended- release formulations. In preclinical diabetes models, exenatide enhanced glucose- dependent insulin secretion, suppressed inappropriately elevated glucagon secretion, slowed gastric emptying, reduced body weight, enhanced satiety, and preserved pancreatic B-cell function. In clinical trials, both exenatide formulations reduced hyperglycemia in patients with type 2 diabetes mellitus (T2DM) and were associated with weight loss. Areas covered: This article reviews the development of exenatide from its discovery and preclinical investigations, to the elucidation of its pharmacological mechanisms of action in mammalian systems. The article also presents the pharmacokinetic profiling and toxicology studies of exenatide, as well as its validation in clinical trials. Expert opinion: GLP-1 receptor agonists represent a new paradigm for the treatment of patients with T2DM. By leveraging incretin physiology, a natural regulatory system that coordinates oral nutrient intake with mechanisms of metabolic control, these agents address multiple core defects in the pathophysiology of T2DM. Studies have identified unique benefits including improvements in glycemic control and weight, and the potential for beneficial effects on the cardiometabolic system without the increased risk of hypoglycemia associated with insulin therapy. Peptide hormone therapeutics can offer significant advantages over small molecule drug targets when it comes to specificity, potency, and more predictable side effects. As exemplified by exenatide, injectable peptides can be important drugs for the treatment of chronic diseases such as T2DM.

Brand Name: (Byetta®)

Generic Name: Byetta and BydureonDosage Form: Injection (Liq)

Treatment**:** used to treat type-2 diabetes

Elimination half-life: ~2.4h

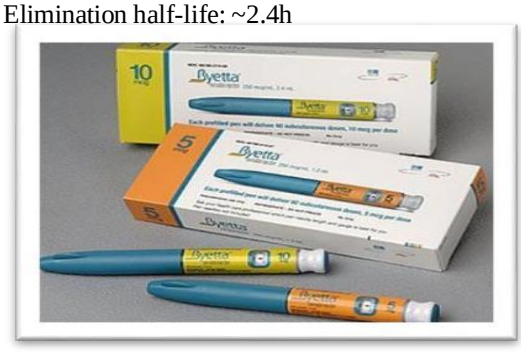


Fig 1: Exenatide

PHASES OF DRUG DEVOLPMENT:

DISCOVERY OF EXENATIDE:

Dr. Eng discovered that exendin-4 could be used to stimulate insulin secretion in various models, he received in 1995. Dr. Eng presented his findings on the long-acting actions of exendin-4 in diabetic mice at the annual meeting of the American Diabetes Association in June 1996. Scientists from various pharmaceutical companies expressed interest in learning more, but it was Amylin Pharmaceuticals, Inc. that jumped at the opportunity. Within months of seeing the poster at the American Diabetes Association meeting. Amylin licensed exendin-4 and began further research into the compound as a potential therapeutic agent .

Dr. Eng found that exendin-4 had glucose-lowering effects, making it potentially useful as a treatment for type 2 diabetes. Exendin-4 shares many of the same properties as glucagon- like peptide-1 (GLP-1), a gut hormone that plays an important role in regulating glucose in humans. Both exendin-4 and GLP-1 enhance the body's abilityto release insulin only in response to elevated levels of glucose, thereby reducing the likelihood that glucose levels will be too high or too low; however, there is an important difference between the two molecules, GLP-1 is metabolized in less than two minutes upon administration, which has frustrated attempts to develop GLP-1 into a viable treatment for diabetes. In contrast, exendin-4 has much longer activity, lasting for hours. This trait gave the compound value as a potential therapeutic agent.

FORMULATION O F EXENATIDE:

The ER formulation of exenatide was developed to provide patients with long-term exenatide exposure allowing for a once-weekly dosing interval. This formulation is based on dispersing exenatide in a structural matrix composed of medical-grade biodegradable poly (D,L-lactide-co- glycolide) (PLGA) polymer. After SC administration, the PLGA polymer undergoes hydrolysis inthe SC space leading to a continuous release of exenatide. The release of exenatide from the PLGApolymers following a single injection results in a total duration of drug exposure of 10-12 weeks. The concentration-time profile of the ER formulation after a single SC injection is characterized by three distinct phases. Phase 1 is an initial release of exenatide that is loosely bound to the surface of the microsphere, which is readily released upon hydration of the microsphere and occurs within the first 24 h after SC administration. Following this initial release, the PLGA polymer is slowly

hydrolyzed leading to a slow release of exenatide, cumulating in a second peak occurring approximately 2 weeks after an SC dose. During the third phase, the microspheres fully hydrolyze and all the remaining drug is slowly released in the SC space to be absorbed into the systemic circulation, producing a third peak that appears in the concentration- time profile approximately 7 weeks postdose. The microspheres themselves are degraded to water an CO2 in the SC space and are not absorbed intact into the circulation. Once released from the microspheres and absorbed into the circulation, the exenatide peptide follows the same distribution and elimination properties as the IR formulation.

The formulation of exenatide following both single- and multiple- dose administration. Such a model must account for each phase of drug release, providing a quantitative platform for assessing the factors influencing the time to steady-state and average plasma drugj concentrations following multiple- dose administration. The final model may be used to test hypotheses pertaining to the influence of intrinsic factors on components of the concentration-time profile and ultimate pharmacodynamic responses, as well as for simulating alternative dosing regimens.

PHARMACOKINETICS ASPECTS AND EXENATIDE DRUGDIPOSITION:

The pharmacokinetics of exenatide BID are dose proportional, with maximum serum concentrations after a single subcutaneous dose of 2.5 or 5 µg of 56 and 85 pg/mL, respectively, and the area under the concentration- time curve of 159 and 340 pg.h/mL. The time to reach maximum serum concentrations after exenatide BID administration is ~2 h.Exenatide QW is the same active drug as exenatide BID dispersed in microspheres made of the medical grade, biodegradable polymer poly-(D,L-lactide-co-glycolide), unlike exenatide BID, which is immediately available and has an elimination half-life of 2.4 h, the peptide dispersed in the microspheres is released via biodegradation in three stages: initial release (first 48 h), diffusion (~2weeks), and erosion release (~7 weeks) Therapeutic delivery from microspheres is unique as,over time, the exenatide dose in a given week is derived from multiple previous injections undergoing different phases of microsphere dissolution With simultaneous release from multiple microspheres at different stages of dissolution,exenatide is released at a constant rate,without peaksor troughs in drug concentration.

ABSORPTION:

The subcutaneous administration to patients with type 2 diabetes, exenatide reaches median peak plasma concentrations in 2.1 h. Mean peak exenatide concentration (Cmax) was 211 pg/mL and overall mean area under the curve (AUCo-int) was 1036 pgh/mL following SC administration of a 10mcg dose of BYETTA. Exenatide exposure (AUC) increased proportionally over the therapeutic dose range of 5 mcg to 10 mcg. The Cmax values increased less than proportionally over the same range. Similar exposure is achieved with SC administration of BYETTA in the abdomen, thigh, or arm.

DISTRIBUTION:

The mean apparent volume of distribution of exenatide following SC administration of asingle dose of BYETTA is 28.3 L.

METABOLISM AND ELIMINATION:

Nonclinical studies have shown that exenatide is predominantly eliminated by glomerular filtration with subsequent proteolytic degradation. The mean apparent clearance of exenatide in humans is 9.1 L/h and the mean terminal half-life is 2.4 h. These pharmacokinetic characteristics of exenatide are independent of the dose. In most individuals, exenatide concentrations are measurable for approximately 10 h post-dose.

P

HARMACODYNAMICS:

Postprandial Glucose

In patients with type 2 diabetes, BYETTA reduces the postprandial plasma glucose concentrations (FIGURE:1).

FIGURE:2 Mean (+SEM) Postprandial Plasma Glucose Concentrations on Day 1 of BYETTA" Treatment in Patients With Type 2 Diabetes Treated With Metformin, a Sulfonylurea, or Both (N = 54)

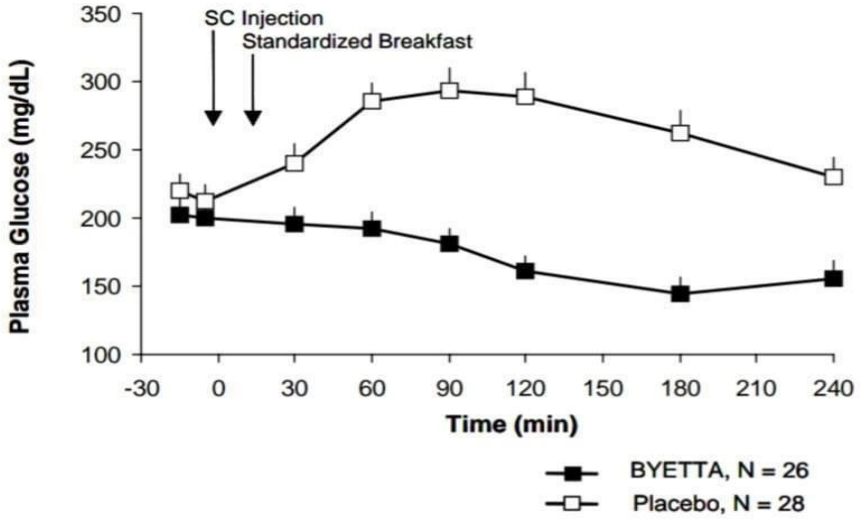


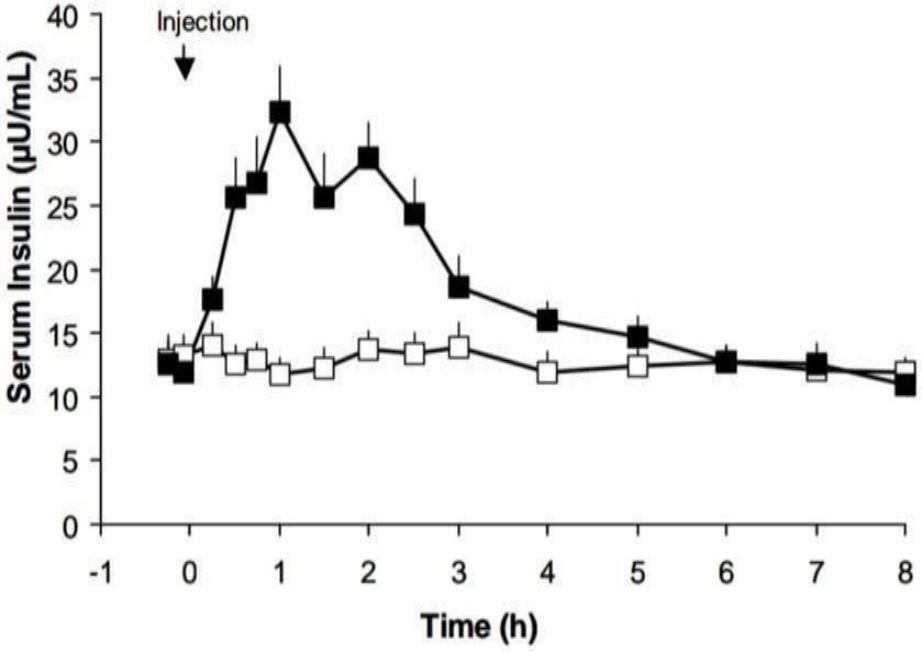
Fig 2: Postprandial glucose

"Mean dose (7.8 meg based on body weight) was administered by subcutaneous (SC)injection.

FASTING G L U C O S E :

In a single-dose crossover study in patients with type 2 diabetes and fasting hyperglycemia, an immediate insulin release followed injection of BYETTA. Plasma glucose concentrations were significantly reduced with BYETTA compared with placebo (Figure 2).

FIGURE:3 Mean (+SEM) Serum Insulin and Plasma Glucose Concentrations Following a One-Time Injection of BYETTA" or Placebo in Fasting Patients With Type 2Diabetes (N = 12)



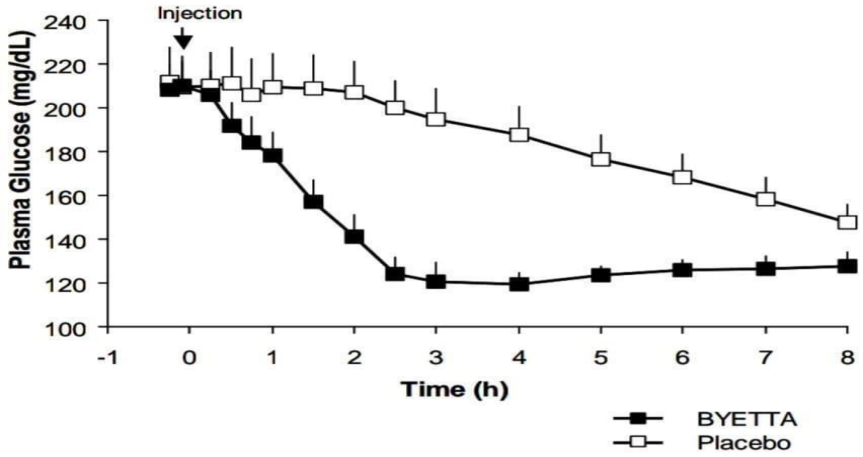


Fig 4: Fasting Glucose

"BYETTA administration was based on body weight at baseline; mean dose was 9.1 mcg.

PRECLINICAL STUDIES AND INVESTIGATIONAL NEW DRUG:

Flavonoids are naturally occurring compounds with several biological effects, such as antioxidant anti-neoplasic anti-inflammatory, anti-hyperglucemic and antihypertensive. These natural products have been identified in different fruits and vegetables, such as tomato, mandarin, grapefruit, lemon and orange Particularly, six major flavonoids are widely distributed in sweet orange species: rutin, quercetin, hesperetin, naringin, herperidin and naringenin.

Early preclinical studies have been conducted where their biological effects are demonstrated. Thus, intragastric administration of naringenin (50 mg/kg) induced a significant decrease in plasma glucose in non-insulin-dependent diabetic rats. The oral administration of hesperidin (200 mg/kg) demonstrated a hypolipidemic effect in cardiotoxicity-induced rats.

In this context, our research group developed a mixture of naringenin-hesperidin, MIX-
160. This preparation demonstrated improved absorption when administered as a mixture (161 mg/kg) compared with a single dose of naringenin (92 mg/kg) and hesperidin (69 mg/kg) [1 Oral administration of MIX-160 produced a significant decrease in systolic and diastolic blood pressure in spontaneously hypertensive rats at 5 and 7 h post-administration. Furthermore, subchronic oral administration of the mixture for 30 days improved carbachol-induced relaxation and exerted less vascular contractibility in norepinephrine induced contraction.

A subchronic toxicity study of methyl hesperidin in B6C3F1 mice at a dose of 5.0% in the diet for 13 weeks revealed no obvious toxic effects in mice of either sex. Peibo Li et al. proved that naringin was practically non-toxic for Sprague-Dawley rats in an oral acute toxicity study andthe no-observed-adverse-effect- level (NOAEL) of naringin in rats was greater than 1250 mg/kg/day when administered orally for 13 consecutive weeks Other results reported by Andrade-Ortiz et al. were also consistent with these data, thus, positioning these flavonoids as low-risk, useful substances for drug development.

HISTORICAL APPROACHES IN DRUG DISCOVERY:

AYURVEDIC:

Breaking the myth that 'Diabetes cannot be cured but only managed', this exclusive and personalized 'Diabetes health program' helps you to combat the menace of Diabetes and comorbidities associated with it while keeping healthy levels of glucose naturally. The program isa very unique and engaging ayurvedic treatment for reversing diabetes makes it possible to live without this condition that keeps you worried all the time when you see your platter and start counting carbohydrates. India being the diabetic capital of the world, faces a substantial economic

burden of Diabetes and other lifestyle disorders with drugs worth billions are consumed by the patients in the hope of managing (Not Curing) their disease. At the same time, they seldom give a thought to its reversal. Surprisingly, it is very much possible with Ayurveda medicines and personalized dietary regimen if planned in sync with the patient's Prakriti which makes Ayurvedic treatment of diabetes more inclusive and effective.

Three step approach Ayurvedic Treatment for diabetes: 1. cleaning phase
2.Increased Body's insulin sensitivity3. Personalized program

1. Cleaning phase:

In Diabetes mellitus, the pancreas does produce Insulin. Still, the body cells are unable to utilize it; it is simply because they are jammed and do not allow Insulin's action on it for glucose to enter in it and utilize it. This inability of your cells makes glucose travel in your blood and hence, a raised blood sugar. While most of the health expert puts you either on anti-diabetic synthetic drugs or starve you, the ayurvedic treatment of 'Shodhan' or cleansing helps your cells tostart processing Insulin. This cleansing prepares you towards a diabetes free life while pacing up the recovery process.

2. Increasing Body's insulin Senstivity:

The diabetes health program helps you in increasing your body's insulin sensitivity/response most healthily without even bad shaming carbohydrates. Eating carbohydrates do not cause Diabetes, it is your body's inability to process sugar or low digestive fire as per Ayurveda what causes you Diabetes. The ayurvedic treatment from Aas Ayurveda is planned in the way that youstill eat carbohydrates, albeit complex ones and help you in reversing it. The regimen also helps in the cases of Diabetes type 1 by stimulating the pancreas, where it has been seen patients curtailingon the use of Insulin and in most cases, stop using it.

3. Personalized program:

Every individual looks differently and behaves differently, and the reason precisely lies in their Prakriti/constitution. We take the help of this Ayurveda Prakriti to plan your dietary regimen that suits your daily schedule, eating habits and most importantly, to your gut. Striving on the pillars of patient education, patient engagement and patient empathy, the regimen.

CHINEASE MEDICINE

The Chinese ginseng Panax notoginseng (Burkill) FH.Chen (Araliaceae) (is probably native to south-eastern China and Vietnam but has spread to forests from China to the Himalayas and Myanmar. P. must not be confused with other Panax species such as the Asianginseng P. ginseng C.A. Meyer and the American ginseng P. quinquefolius L., which it superficially resembles. However, an important distinguishing characteristic of P. notoginseng is the presence of three petioles with seven leaflets each. This is the reason this plant is referred to in China as "sān-qī," meaning "the three-seven herb." P.notoginseng is either cultivated or gathered from the wild, and the interest in this plant is particularly for its root and rhizome which are used to prepare foods, health products, beauty products, dietary supplements, an medicines.

NEW APPROACHES IN DRUG DISCOVERY

Currently, the strategies to achieve outcomes described in the TPP use islet encapsulation approaches in a variety of ways: (a) mechanical modification, resulting in changing factors such as capsule size, surface charge, mechanical strength, permeability, and material biocompatibility,

(b) chemical modification, through the inclusion of immunomodulatory factors including anti- inflammatory agents, targeted immunosuppressant pharmaceuticals, and anticoagulant materials;

(c) co-encapsulations with biologicals such as bioengineered cells, chemokines, ECM proteins, and angiogenic factors; and (d) co-encapsulations with chemical agents. However, to date, none of these. strategies alone have been successful at achieving all cri teria outlined in the TPP. In this section, for each clinical outcome in the TPP. we have highlighted three to five key preclinical studies that combine encapsulation approaches to better achieve ideal clinical outcomes. The studies were chosen based on the availability of the methodology as well as the primary metric used in each category (e.g. survival rates for viability of islets and insulin independence).

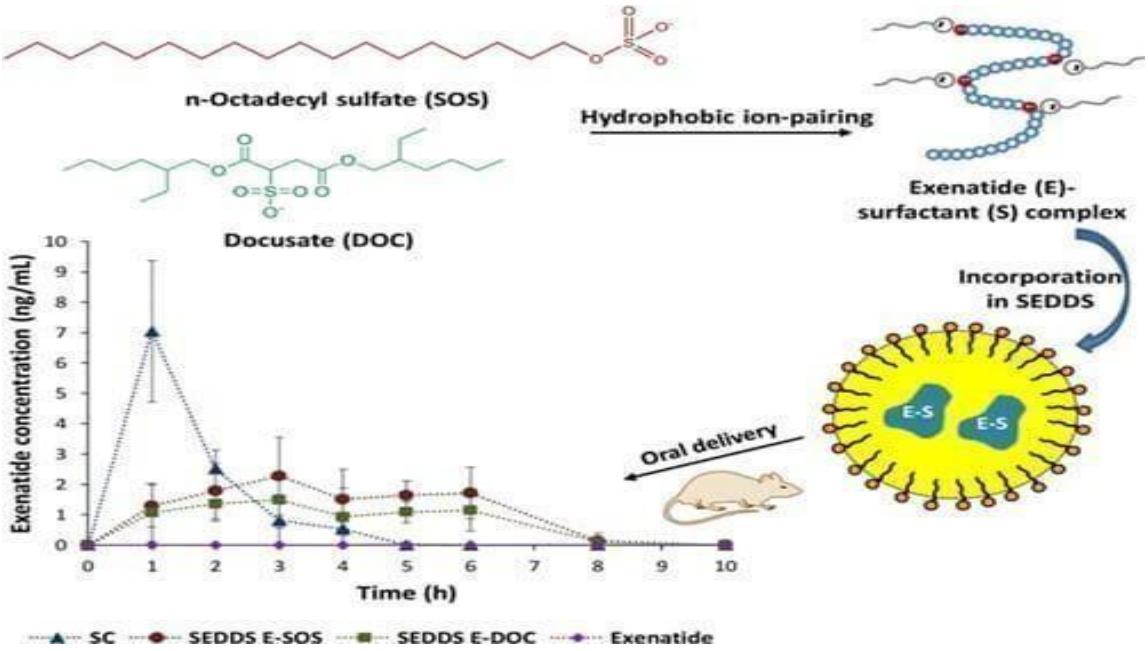


Fig 5: Combinotorial Chemistry

ULTRA-HIGH-THROUGHPUT S C R E E N I N G :

The trend towards assay miniaturization for high-throughput and ultra-high-throughput screening continues to spur development of homogeneous, fluorescence-based assays in higher density smaller volume microplate formats. Recently, first-generation microfluidic devices have been designed for performing continuous-flow biochemical and cell-based assays. These devices provide orders-of-magnitude reduction in reagent consumption, and offer the potential for implementing high-throughput screening in formats that integrate up-front compound handling with unique assay functionality.

Over the past decade, a variety of scientific advances and economic pressures have driven the need for improved drug discovery screening technology 1, 2. These include the growing number of potential therapeutic targets emerging from the field of functional genomics, the rapid development of large compound libraries derived from parallel and combinatorial chemical synthesis techniques, and the ever increasing pressure to reduce development costs while enhancing commercial competitiveness in the pharmaceutical industry. Recent estimates of the number of individual genes in the human genome (~10,000) and the number of unique chemical structures theoretically attainable using existing that up to 1012 assays would be required to completely map the structure-activity space for all potential therapeutic targets.

THE COMMITTEE FOR THE PURPOSE OF CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS GUIDELINES FOR THE CARE AND USE OF LABARATORY ANIMALS (CPCSEA)

GOAL

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioral 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 that is relevant to humans and animals.

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, mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behavior ,and well-being is conveyed to the attending veterinarian. The veterinarian can also contribute to the establishment of appropriate policies and procedures for ancillary aspects of veterinary care,

such as reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

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 large animals is 6 weeks (cat, dog and monkey) Effective quarantine procedures should be used for non-human primates to help limit exposure of humans zoonotic infections. Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychological and nutritional stabilization before their use. 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 physiological and behavioral changes due to interspecies conflict. Such separation is usually accomplished by housing different species in separate rooms; for example ,if two species have a similar pathogen status and are behaviourly compatible.

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

All animals should be observed for signs of illness ,injury, or abnormal behavior by animal house staff. Asa rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis (Annexure 1 and 2).Unexpected deaths and signs of illness, distress, o rother deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care.

Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious agent (e.g. Mycobacterium Tuberculosis in non-human primates), the group should be kept intactand isolated during the process of diagnosis, treatment, and control. diagnostic ,treatment and control. Diagnostic clinical laboratory may be made available.

ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

Institutions should have policies governing experimentation with hazardous agents. Institutional Biosafety Committee whose members are knowledgeable about hazardous agents arein place in most of the higher level education, research institutes and in many pharmaceutical industries for safety issues. This committee shall also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions (Annexure-8).Since the use of animals in such studies requires special consideration, the procedures and the facilities to be used must be reviewed by both the Institutional Biosafety Committee and Institutional Animal Ethics Committee (IAEC)

DURATIONS OF EXPERIMENTS

No animal should be used for experimentation for more than 3 years unless adequate justification is provided..

PHYSICAL RESTRAINT

Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injuryto the animal .Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to research objectives. Less restrictive systems, such as the tether system or the pole and collar system, be used when compatible with research objectives. The following are important guidelines for the use of restraint equipments:

Restraint devices cannot be used simply as a convenience in handling or managing animals. The period of restraint should be the minimum required to accomplish the research objectives. Animals to be placed in restraint devices should be given training to adapt to the equipment

.Provision should be made for observation of the animal at appropriate intervals. Veterinary care should be provided if lesions or illness associated with restraint are observed. The presence of lesions, illness, or severe behavioral change should be dealt with by the temporary or permanent removal of the animal from restraint.

PHYSICAL FACILITIES

(a) Building materials :should be selected facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.
(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 pro(b) Corridor(s): should be wide enough to facilitate the movement of personnel as well as equipments and should be kept clean.
They Should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.
(e) Exterior windows :Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate sources of light and ventilation. In primate rooms, windows can be provided.
(F) Floors :Floors should be smooth, moisture proof, nonabsorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants. They should be capable of supporting racks ,equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints. A continuous moisture-proof membrane might be needed. If sills are installed at the entrance to a room ,they should be designed to allow for covenient passage of equipment.
(g) Drains :Floor 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. Where floor drains are used, the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying of surfaces.
(h) Walls and ceilings: Walls should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors ,ceilings, floors and corners. Surface materials should be capable of withstanding scrubbing with detergents and disinfectants and the impact of water under high press.

ENVIRONMENT

(a) Temperature and humidity control Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions or differences in the number and kind of room occupants. Ideally, capability should be provided to allow variations within the range of approximately 18 to 29°C (64.4 to 84.20F), which includes the temperature ranges usually recommended for common laboratory animals. The relative humidity should be controllable within the range of 30% to 70% throughout the year. For larger animals a comfortable zone (18 to 37°C) should be maintained during extreme summer by appropriate methods for cooling.
(b) Ventilation In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals' primary enclosures. Heating, ventilating, and air- conditioning systems should be designed so that operation can be continued with a standby system. The animal facility and human occupancy areas should be ventilated separately.
(c) Power and lighting The electrical system should be safe and provide appropriate lighting and a sufficient number of power outlets. It is suggested that a lighting system be installed that provides adequate illumination while people are working in the animal rooms and a lowered intensity of light for the animals. Fluorescent lights are efficient and available in a variety of acceptable fixtures. Atime-controlled lighting system should be used to ensure a regular diurnal lighting cycle wherever required. Emergency power should be available in the event of power failure.
(d) Noise control The facility should be provided with noise free environment. Noise control is an important consideration in designing an animal facility. Concrete walls are more effective than metal or plaster walls in containing noise because their density reduces sound transmission.

ANIMAL HUSBANDRY

(a) 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 well being, meet research requirements, and minimize experimental variables. The housing system should: y provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species; (Annexure - 3) ÿ provide a comfortable environment yprovide an escape proof enclosure that confines animal safety y provide easy acces to food and water; y provide adequate ventilation y meet the biological needs of the animals, e.g., maintenance of body temperature, urination, defecation and reproduction.

STANDARD OPERATING PROCEDURES (SOPs) /GUIDELINES

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: ÿ Name of the Author ÿ Title of the SOP ÿ Date of preparation ÿ Reference of previous SOP on the same subject and date (Issueno and Date) ÿ Location and distribution of SOPs with sign of each recipient ÿ Objectives ÿ Detailed information of the instruments used in relation with animals with methodology (Model no., Serial no. and Date of commissioning) ÿ The name of the manufacturer of the reagents and the methodology of the analysis pertaining to animals y Normal value of all parameters y Hazard identification and risk assessment.

PERSONNEL AND TRAINING

The selection of animal facility staff, particularly the staff working in animal rooms or involved in transportation, is a critical component in the management of an animal facility. The staff must be provided with all required protective clothing (masks, aprons, gloves and gumboots and other footwear) while working in animal rooms. Facilities should be provided for change over with lockers, wash basin, toilets and bathrooms to maintain personal hygiene. It is also important a regular medical check-up is arranged for the workers to ensure that they have not picked up anyzoonotic infection and also that they are not acting as a source of transmission of infection to theanimal

SPECIAL ARTICLE

In-charge should ensure that persons working in animal house do not eat, drink, smoke inanimal room and have all required vaccination, particularly against tetanus and other zoonotic

diseases. Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the animal handling techniques, cleaning of cages importance of hygiene, disinfection and sterilization. They should also be made familiar with the activities of normal healthy and sick animals so that they are able to spot the sick animal during their daily routine check up of cages. 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 thermo- regulatory mechanism in addition to central nervous system. Before using actual anaesthetics the animal is prepared for anaesthesia by overnight fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardio-pulmonary system and reduce salivary secretion. Atropine is the most used anticholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure. Local anaesthetics are used to block the nerve supplyto a limited area and are used only for minor and rapid procedures.

EUTHANSIA

Euthanasia is resorted to events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons, procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety For accepting an 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 (Annexure-6). The method should in all cases meet the following requirement.

- Death, without causing anxiety, pain or distress with minimum time lag phase.
- Minimum physiological and psychological disturbances.
- Compatibility with the purpose of study and minimum emotional effect on the operator.
- location should be separate from animal rooms and free from environmental contaminants. Tranquilizers have to be administered to larger species such as monkeys, dogs and cats before an euthanasia procedure.

PRECLINICAL STUDIES

Exenatide has been reported to exhibit a number of acute and longer-term actions in animal modelsof type 2 diabetes that may account for its glucose-lowering effects in humans. A review of the effects of exenatide in nonhuman models of diabetes is provided by Nielsen et al and is on- ly briefly summarized here. Exenatide has demon- strated dose-dependent reductions in plasma glucose in mice, rats and monkeys and the activity was glucose dependent. In rat models, exenatidereduces HbA1c and increases insulin sensitivity . Arecent study in a rat model of insulin resistancehas shown that exenatide exhibits an insulin-sensitizing effect that is partially, but not fully, attributable to changes in food intake and largely independent of glycemic improvement. Glucose-dependent insulinotropic effects of exenatide have been demonstrated in mice and rats . A number of studies provide strong evidence that exenatide

plays an important role in the maintenance of beta-cell mass and function through stimulation of islet During the treatment period, the rats in normal control group were in good condition, with glossy fur, free movement and sensitive reaction. In model group. the rats had obviously poor mental state. The fur gradually lost luster. The movement and response were slow. Compared with model group, the symptoms of rats in 3 exenatide groups were mild, especially in exenatide-2 and exenatide-3 groups. There was no rat dying in each group during the experiment -ll proliferation and neogenesis, and inhibition of islet cell apoptosis . Using a rat model of intrauterine growth retardation, Stoffers et al. have addressed the question of whether exenatide can prevent the development of diabetes . Exenatide was administered for 6 days following birth and resulted in markedly reduced levels of fasting glucose and improved glucose tolerance even at 3 months of age in association with highly significant increases in beta-cell mass . Exenatide treatment has been associated with dose-dependent reductions in food intake and weight loss in experiments in rat models of diabetes which re sustained over long-term treatment.

TOXICITY STUDIES
ACUTE TOXICITY
Single-dose toxicity studies were conducted in mice, rats, and monkeys. No lethality or serioustoxicity was observed in mice, rats, or monkeys at doses up to 1500 µg/kg (intravenous). 30,000 µg/kg (subcutaneous), or 5000 µg/kg (subcutaneous) respectively.

Repeat-Dose Toxicity
Repeat-dose toxicity studies were conducted in mice, rats, and monkeys. Decreased body weight gain and food consumption, a known pharmacologic effect of exenatide, were observed in all repeat-dose toxicity studies. No target organ toxicities occurred in mice, rats, or monkeys at subcutaneous doses up to 760 ug/kg/day (j182 days), 250 µg/kg/day (91 days), or 150 ug/kg/day (273 days), respectively, with corresponding systemic exposures of up to 519, 128, and 482 times the human exposure resulting from the maximum recommended dose of 20 µg/day based on plasma area under the curve (AUC), respectively.

Carcinogenicity
A 104-week carcinogenicity study was conducted in male and female rats at doses of 18, 70, or 250 µg/kg/day administered by bolus subcutaneous injection. An apparent numerical increase in benign thyroid C-cell adenomas was observed in female rats given the high dose of 250 µg/kg/day, a systemic exposure 130 times the human exposure resulting from the maximum recommended dose of 20 ug/day, based on AUC. This increased incidence was not statistically significant when adjusted for survival. There was no tumorigenic response in malerats.

Mutagenicity
Exenatide was not mutagenic or clastogenic, with or without metabolic activation, in the Ames bacterial mutagenicity assay or chromosomal aberration assay in Chinese hamster ovary cells. Exenatide was negative in the in vivo mouse micronucleus assay.

Impairment of Fertility
mouse fertility studies with subcutaneous doses of 6, 68 or 760 µg/kg/day, males were treated for 4 In weeks prior to and throughout mating and females were treated 2 weeks prior to and throughout mating until Gestation Day 7. No adverse effect on fertility was observed at 760 µg/kg/day, a systemic exposure 390 times the human exposure resulting from the maximum recommended dose of 20 ug/day, based on AUC.
Teratogenicity

In pregnant mice given subcutaneous doses of 6, 68, 460, or 760 µg/kg/day from Gestation Day 6 through 15 (organogenesis), fetal growth was slowed at doses 268 µg/kg/day exenatide (223 times the human exposure). Administration of higher doses of exenatide (460 µg/kg/day) was associated with skeletal effects known to be associated with slowed fetal growth. The NOAEL for developmental effects in mice was 6 µg/kg/day (3 times the human exposure, based on AUC). In pregnant mice given subcutaneous doses of 6, 68, or 760 µg/kg/day from Gestation Day 6 through Lactation Day 20 (weaning), slowed neonatal growth was observed in the F1 offspring at doses 268 µg/kg/day (223 times the human exposure resulting from the maximum recommended of 20 µg/kg/day, based on AUC). Increased perinatal and neonatal mortality occurred in the F1 offspring at 760 ug/kg/day (390 times the human exposure resulting from the maximum recommended dose of 20 ug/kg/day, based on AUC). The NOAEL for developmental toxicity in mice was 6 µg/kg/day (3 times the human exposure, based on AUC). In pregnant rabbits given subcutaneous doses of 0.2, 2, 22, 156, or 260 µg/kg/day from Gestation Day 6 through 18 (organogenesis), fetal growth was slowed at doses greater than or equal to 22 µg/kg/day (2207 times the human exposure resulting from the maximum recommended dose of 20 µg/day, based on AUC). The NOAEL for developmental effects in rabbits was 2 µg/kg/day (12 times the human exposure, based on AUC).

ANIMAL MODELS IN PRECLINICAL R E S E A R C H :

Fifty male Sprague Dawley rats (200+30 g) were adaptively fed for 1 week. Then, the rats were divided into normal control, model, low-dose exenatide (exenatide-1), middle-dose exenatide (exenatide-2) and high-dose exenatide (exenatide-3) groups according to random number table, with 10 rats in each group. The rats in model and 3 exenatide groups were fasted for 12h, followed by single sterile intraperitoneal injection of streptozotocin with dose of 60 mg/kg. After 72h, the tail vein blood was sampled, and the fasting blood glucose (FBG) level higher than 16.7 mmol/L indicated the diabetes. After 3 weeks, the 24-hours urine protein (24h UP) was detected. The 24h UP higher than 30 mg indicated the diabetic nephropathy In this study, the diabetic nephropathy model was successfully constructed in 40 rats.

General condition of Rats

During the treatment period, the rats in normal control group were in good condition, with glossy fur, free movement and sensitive reaction. In model group. the rats had obviously poor mental state. The fur gradually lost luster. The movement and response were slow. Compared with model group, the symptoms of rats in 3 exenatide groups were mild, especially in exenatide-2 and exenatide-3 groups. There was no rat dying in each group during the experiments.

Effects of exenatide on body weight of rats

At the end of treatment, the body weight of rats in normal control group was 442.34+58.12 g. The body weight in model, exenatide-1, exenatide-2 and exenatide-3 groups were 277.62+42.33 g, 288.14+36.29 g, 311.58-45.48 g and 334.04+57.46 g, respectively, which was significantly lower than normal control group, respectively (P<0.05). There was no significant difference of body weight among model group and 3 exenatide groups (P>0.05) (Figure:6)

Figure:1 Body weight of rats in different groups:

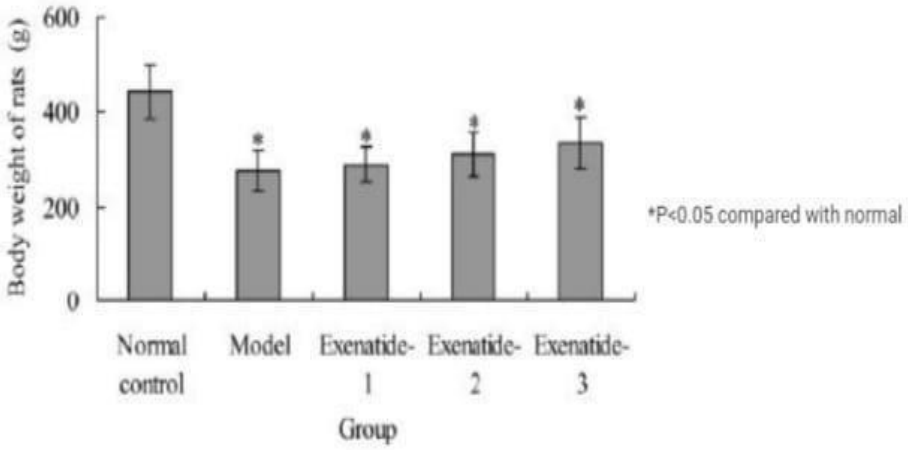


Fig 6: Effects of rats

Effects of exenatide on glucose metabolism indexes of rats

After treatment, compared with the normal control group, in model group and 3 exenatidegroups the FBG and HbA1 levels were significantly increased, respectively ($P < 0.05$), a the FINS level was significantly decreased, respectively ($P<0.05$). Compared with the modelgroup, the FBG and HbA1 levels in exenatide- 2 and exenatide-3 groups were significantly decreased, respectively ($P < 0.05$), and the FINS level in exenatide-3 group was significantlyincreased ($P < 0.05$) (Table 1).

Table 1 Glucose metabolism indexes of rats in different groups:

Group	FBG (mmol/L)	FINS (μg/L)	HbA1 (%)
Normal control	5.52±1.06	0.88±0.12	4.33±0.74
Model	20.33±3.12*	0.55±0.08*	10.48±1.85*
Exenatide-1	19.48±2.87*	0.56±0.07*	10.08±1.72*
Exenatide-2	17.36±2.73*#%	0.62±0.08*	8.12±1.98*#%
Exenatide-3	17.04±2.56*#%	0.73±0.09*#%&	8.05±1.44*#%

TABLE:1

Effects of exenatide on renal function indexes of rats

Table 2 showed that, after treatment, compared with the normal control group, the renal index and Scr level in mode, exenatide-1 and exenatide-2 groups and BUN and 24 h UP levels in model and 3 exenatide groups were significantly increased, respectively ($P<0.05$). Compared with the model group, in exenatide and exenatide-3 groups the renal index, BUN, Ser and 24h UP levels were significantly decreased, respectively ($P<0.05$).

Table:2 Renal function indexes of rats in different groups:

Group	Renal index (mg/g)	BUN (mmol/L)	Scr (μmol/L)	24h UP (mg)
Normal control	1.41±0.17	6.82±1.45	22.46±2.58	6.47±1.26
Model	2.18±0.22*	11.52±1.98*	28.83±3.07*	62.73±11.73*
Exenatide-1	2.11±0.28*	10.77±1.46*	27.28±2.72*	58.29±10.63*
Exenatide-2	1.92±0.26*#%	9.02±1.07*#%	25.78±2.62*#%	42.63±8.51*#%
Exenatide-3	1.61±0.23#%&	8.82±1.97*#%	24.19±2.62#%&	34.68±7.27*#%&

*P < 0.05 compared with normal control group; #P < 0.05 compared with model group; %P < 0.05 compared with exenatide-1 group; &P < 0.05 compared with exenatide-2 group. BUN, blood urea nitrogen; Scr, serum creatinine; 24h UP, 24-hour urine protein.

TABLE:2

Effects of exenatide on renal tissue oxidative stress indexes of rats

After treatment, compared with the normal control group, in mode, exenatide-1 and exenatide-2 groups the renal tissue SOD and GSH-Px levels were significantly decreased, respectively ($P < 0.05$), and the renal tissue MDA level was significantly increased, respectively ($P < 0.05$). Compared with model group, the SOD level in exenatide-2 and exenatide 3 groups was significantly increased, respectively ($P < 0.05$), the GSH-Px level in exenatide-3 group was significantly increased ($P < 0.05$) and the MDA level in exenatide-2 and exenatide-3 groups was significantly decreased, respectively ($p<0.05$).

Table:3 Renal tissue oxidative stress indexes in different groups.

Group	SOD (U/mg prot)	GSH-Px (U/mg prot)	MDA (nmol/mg prot)
Normal control	193.36±21.32	25.44±3.28	0.79±0.57
Model	133.59±15.34*	18.12±2.56*	1.25±0.21*
Exenatide-1	136.17±16.12*	18.67±3.12*	1.23±0.15*
Exenatide-2	156.66±19.89*#%	19.47±4.04*	1.05±0.19*#%
Exenatide-3	178.131±19.71#%&	23.72±3.56#%	0.98±0.22#%

*P < 0.05 compared with normal control group; #P < 0.05 compared with model group; %P < 0.05 compared with exenatide-1 group; &P < 0.05 compared with exenatide-2 group. SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

TABLE:3

Effects of exenatide on renal tissue inflammatory indexes of rats

As shown in Table 4, after treatment, compared with the normal control group,in model group and3 exenatide groups the renal tissue TNF-a, IL-6, hs-CRP andCCL5 levels were significantly increased, respectively ($P < 0.05$) Compared withthe model group, the renal tissue IL-6 level in exenatide-1, exenatide 2 andexenatide-3 groups was significantly decreased, respectively ($P<0.05$)and the renal tissue TNF-a, hs-CRP and CCL5 levels in exenatide -2 and exenatide -3

significantly decreased, respectively ($P < 0.05$).

Group	TNF-α (ng/L)	IL-6 (ng/L)	hs-CRP (mg/L)	CCL5 (ng/L)
Normal control	67.44±11.06	78.29±10.73	38.46±6.12	55.46±7.78
Model	113.37±14.67*	125.36±10.37*	67.84±8.44*	98.84±9.84*
Exenatide-1	109.21±15.32*	108.63±11.29*#	63.63±7.85*	90.63±9.12*
Exenatide-2	95.56±10.14*#	102.12±14.83*#	56.72±7.13*#	76.72±8.06*#%
Exenatide-3	79.73±9.86*#%&	93.29±9.18*#%	47.39±6.36*#%	69.39±7.39*#%

*P < 0.05 compared with normal control group; #P < 0.05 compared with model group; %P < 0.05 compared with exenatide-1 group; &P < 0.05 compared with exenatide-2 group. TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; hs-CRP, hypersensitive C-reactive protein; CCL5, chemokine (C-C motif) ligand 5.

TABLE:4

BIOSTATISTICS IN PRECLINICAL STUDIES

Biostatistics in order to statistically analyze and interpret the data from your in vitro and vivo experiments. conducted to preclinical in Experiments answer one or are more specific scientific questions, and they must be designed so that they are likely to provide answers with minimal bias and appropriate measures of variability and significance. Here, we discuss different methods of analysis and their accompanying assumptions. In addition, we cover several different experimental design considerations as well as the subsequent interpretation and graphical presentation of dataand statistical findings. Furthermore, we provide insight on both sides of the debates surrounding controversial issues such as testing multiple hypotheses in a single study and addressing outliers in the data. We conclude with a discussion of the future of biostatistics for invitro and preclinical experiments, highlighting the importance of learning biostatistical software in your training. We suggest you read this chapter before you begin performing experiments and collecting data.

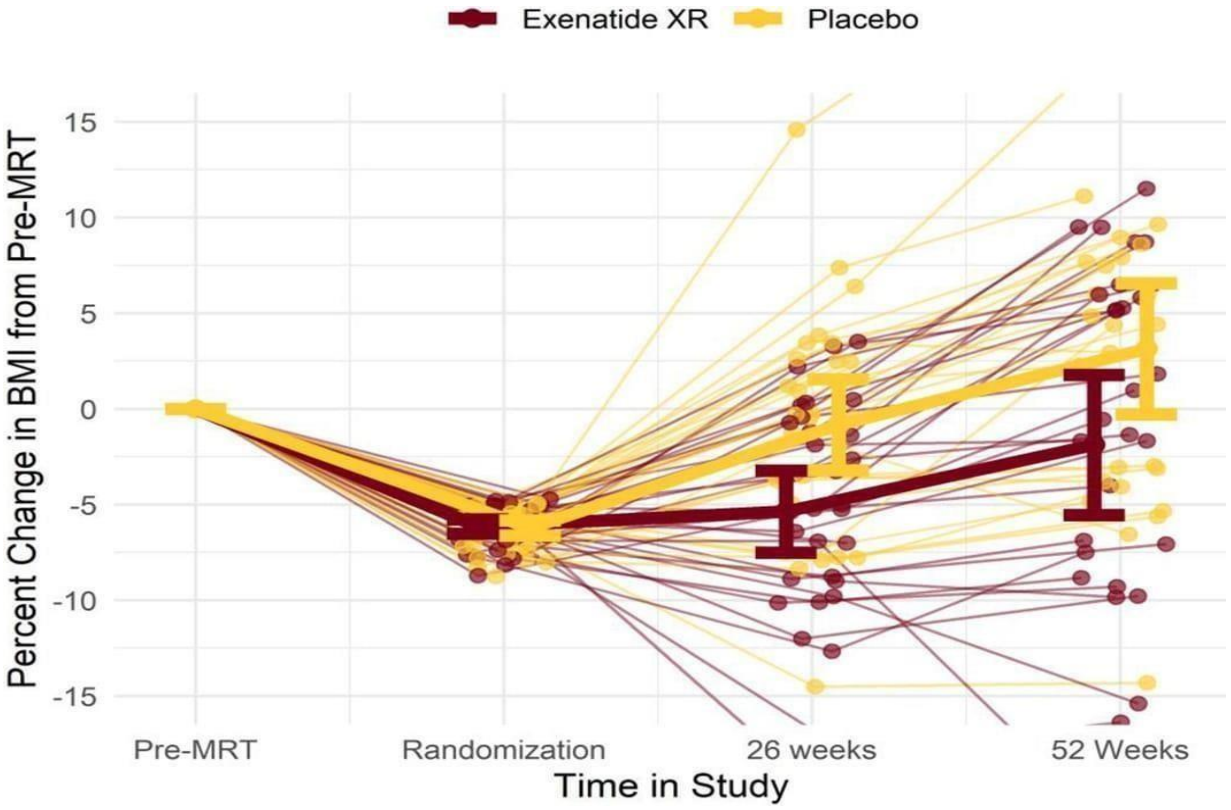


Fig 7: Biostatics in preclinical research

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