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BIOLOGICAL SCREENING PROCEDURES FOR INDIAN HERBAL ANTI-DIABETIC DRUGS

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ABSTRACT

Traditional Medicines derived from medicinal plants are used by about 60% of the world's population. This study focuses on Indian Herbal drugs and plants used in the treatment of diabetes and their biological screening procedures. Diabetes is an important human ailment afflicting many from various walks of life in different countries. In India it is proving to be a major health problem, especially in the urban areas. A number of medicinal plants have proven antidiabetic and related beneficial effects and of herbal drugs used in treatment of diabetes is compiled. These include, *Allium sativum*, *Eugenia jambolana*, *Momordica charantia*, *Ocimum sanctum*, *Phyllanthus amarus*, *Pterocarpus marsupium*, *Tinospora cordifolia*, *Trigonella foenum graecum* and *Withania somnifera*. Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Appropriate experimental models are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of type 2 diabetes and testing of various therapeutic agents. The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and/or by combination thereof. This article gives an overview on the animal models of type 2 diabetes with reference to their source, characteristic features, underlying principal and procedure to the investigators in diabetes research. Models For Insulin Dependent Diabetes Mellitus [IDDM] such as Alloxan induced diabetes, Streptozotocin induced diabetes, Virus induced diabetes, Insulin antibodies induced diabetes, Hormone induced diabetes have given broad spectrum for the evaluation of the anti-diabetic activity, each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying aetiopathogenic mechanisms that could also operate during the evolution of type 2 diabetes in humans. Herbal drugs have values; but they need to go through clinical trials and then only they should be used. Otherwise they can be a menace.

Key words: Anti-diabetic activity, Herbal Drugs, Oral hypoglycaemic agents, Streptozotocin, Alloxan.

INTRODUCTION

Diabetes is one of the leading causes of death in humans and animals. In animals it occurs most frequently in the dog with an incidence of approximately 0.2%.Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world.

It is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus search for a new class of compounds is essential to overcome diabetic problems. Management of diabetes without any side effect is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore it is prudent to look for options in herbal medicines for diabetes as well. Although, herbal medicines have long been used effectively in treating diseases in Asian communities and throughout the world. The mechanism of most of the

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herbals used has not been defined. Many traditional plants treatments for diabetes are also used. But most of the evidence for their beneficial effects is anecdotal [1]. Traditional antidiabetic plants might provide new oral hypoglycemic compounds, which can counter the high cost and poor availability of the current medicines/ present day drugs for many rural populations in developing countries. India is well known for its herbal wealth. Medicinal plants like *Trigonella foenum graecum, Allium sativum, Gymnema slyvestre and Syzigium cumini* have been studied for treatment of diabetes mellitus. However, detailed studies on the efficacy, mechanism of action and safety of plant extract are needed [2]. In the indigenous Indian system of medicine good numbers of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and active principle were isolated. The ethnobotanical information reports state that about 800 plants may possess antidiabetic potential .Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research.

Appropriate experimental models are essential tools for understanding the pathogenesis, complications, and genetic or environmental influences that increase the risks of type 2 diabetes and testing of various therapeutic agents. The animal models of type 2 diabetes can be obtained either spontaneously or induced by chemicals or dietary or surgical manipulations and/or by combination thereof. This review gives an overview on the animal models of type 2 diabetes with reference to their source, characteristic features, underlying principal and procedure to the investigators in diabetes research [2,3].

SCREENING METHODS FOR EVALUATION OF ANTIDIABETIC ACTIVITY OF HERBAL DRUGS

Animals Used For The Screening Of Anti-Diabetic Drug [4,5]

Obese mouse , Diabetic mouse , Sand mouse [Psammomys obesus] ,Spiny mouse [Acomys cahirinus] , BB rats , KK mouse , Yellow mouse , Yellow KK mouse ,New Zealand obese mouse , Tuco-tuco [clenomys talarum], Chinese hamster [Cricetulus griseus] ,NOD mouse Japanese wistar rat [Goto rat] etc.

Chemical Agents Capable Of Inducing Diabetes [5-7]

A) Irreversible beta cytotoxic agents: Alloxan, Steptozocin, Diphenyl thiocarbazine ,Oxine-9- hydroxyquinolone, Vacor.

B) Reversible beta cytotoxic agents 6- aminonicotinamide, l-asparginase , Azide ,Cyanide , Cyproheptadine ,Thiazides Malonates.

C) Other agents - Anti insulin antibodies, Somatostatins, Catecholamines,Glucocorticoids, Glucagoan.

Models For Insulin Dependent Diabetes Mellitus [IDDM] [1-3,5,8-10]

1. Alloxan induced diabetes

Principle

- Alloxan: is a cyclic urea compound, which induces permanent diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells & causes cell death.
- When islets are exposed in vitro to alloxan, it exhibits exceptional beta cell specificity, the other islets cells remaining largely unaffected by both it's inhibitory and cytotoxic effects.

Dose: - In rats Alloxan at dose of 100 mg/kg produces diabetes.

In rabbits dose of 150 mg/kg infused through marginal ear vein produces diabetes in 70% of the animals.

Procedure

- Inject intraperitonially a single dose of alloxan monohydrate [100 mg/kg body weight] dissolved in normal saline to the Albino rats of either sex [150-200g]
- Keep the animals for 48 hours during which food and water is allowed ad libitum.
- Blood glucose levels show triphasic response with hyperglycemia for one hour followed by hypoglycemia that lasts for six hours & stable hyperglycemia after 48 hours.
- Animals showing fasting blood glucose level above 140 mg/dl after 48 hour of alloxan administration are considered diabetic.
- Administered orally the drug samples to be screened for a period of six weeksAfter six weeks of treatment, blood samples are collected from 8 hour fasting animals through a caudal vein.
- Separate the Serum by centrifuge (3000 rpm) under cooling (2-4 °C) for ten minutes.
- Estimate the serum glucose level is by glucose oxidase-peroxidase method [GOD-POD kit] using autoanalyser.

2. Streptozotocin induced diabetes

Principle

Streptozotocin is a broad-spectrum antibiotic, which causes beta islet cell damage by free radical generation. It induces diabetes in almost all species of animals excluding rabbits and guinea pigs.

Diabetes can be induced by Streptozotocin when it is given either as single large (as with alloxan) or as multiple sub diabetogenic injections.

Procedure

- Prepare Streptozotocin [60 mg/kg body weight] in citrated buffer [pH 4.5]
- Inject Albino rats of either sex weighing 150-200 g i.p with above solution.
- Animals showing fasting blood glucose levels > 140mg/dl after 48 hours of streptozotocin administration are considered diabetic.
- After six weeks of treatment blood samples should be collected from 6 hr fasted animals through caudal vein

- Separate Serum is by centrifuge (3000 rpm) under cooling (2-4 °C) for ten minutes.
- Estimate Serum glucose level by glucose- peroxidase method [GOD-POD kit] using autoanalyser.

3. Virus induced diabetes

Viruses are one of the etiological agents for IDDM. They produce diabetes mellitus by infecting and destroying beta cells of pancreas.

Various human viruses used for inducing diabetes include RNA picornovirus, encephalomyocarditis [EMC-D], coxsackie B4 [CB-4].

Procedure

- Inoculate 6-8 week old mice by 0.1 ml of 1:50 dilutions of D-variant encephalomyocarditis [EMC] through i.p.
- 0.1ml of above dilution contains 50 PFU [plaque forming units] of EMC virus.(mortality due to this concentration of virus is approximately 10-20%).
- A less infecting variant produces a comparable damage by eliciting autoimmune reactivity to the beta cells.
- Infected animals are considered hyperglycemic if there non fasting blood glucose levels exceed by 250mg/dl compare to the levels of uninfected animals of the same strain.
- Administer drug samples to be screened orally for a period of 6 weeks.
- After 6 weeks of drug treatment, blood glucose estimation should be done to determine the anti diabetic activity.

4. Insulin antibodies induced diabetes

Principle: - A transient diabetic syndrome can be induced by injecting guinea pigs with anti-insulin serum. Diabetes persists as long as antibodies are capable of reacting with insulin remaining in the circulation.

Preparation of antibody

Dissolve Bovine insulin, in acidified water [ph 3.0] at a dose of 1mg is injected to guinea pigs weighing 300-4oo gm. Collect anti insulin sera after two weeks of antigenic challenge.

Procedure

- Inject Adult albino rats 0.25-1.0 ml of guinea pig anti-insulin serum.
- Insulin antibodies induce a dose dependent increase of blood glucose level up to 300 mg/ dl.
- Administer the drug sample to be screened by a suitable root and analyse blood glucose level to determine the activity.

5 .Hormone induced diabetes

Principle: - Dexamethasone : a steroid possessing immunosuppresion action, which causes an autoimmune reaction in the islets and produces type 1 diabetes.

Procedure

- Inject Adult rats weighing 150-200 gm with dexamethasone at a dose level of 2-5 mg/kg body weight i.p twice a day.
- Repeated injection of same dose level is carried out for a period of 20-30 days resulting in IDDM.
- Administer the sample to be screened through a suitable root.
- Analyse the blood glucose to determine the activity.

Models For NIDDM [2,7,11-15,16-23]

1. Streptozotocin induced neonatal model for NIDDM

Streptozotocin causes severe pancreatic beta cells destruction, accompanied by decrease in pancreatic insulin stores and rise in plasma insulin levels.

Procedure

- Treat Neonatal rats with streptozotocin [90 mg per kg body weight] which should be prepared in citrate buffer [pH 4.5] by i.p at birth or within the first five days following birth.
- Rats develops symptoms similar to NIDDM after six weeks
- Rats showing fasting blood glucose level above 140 mg/ dl are considered diabetic.
- Further steps are similar to alloxan induced diabetes model.
- Administer drug sample to be screened by a suitable route and analyze blood glucose level to determine the activity.

2 .Adrenaline induced acute hyperglycemia

Adrenaline is a counter regulatory hormone to insulin. It increases the rate of glyconeolysis and the glucose levels in blood causing hyperglycemia.

Procedure

- Inject Adult albino rats at a dose level of 0.1 mg / kg through s.c. route
- The dose produces peak hyperglycemic effect after one hour and lasts upto four hours.
- Administer the drug sample to be analysed through a suitable route.
- Determine the blood glucose (The oral hypoglycemic agents can be screened by this method)

3. Dithizone induced diabetes

Principle

Organic agents react with zinc in islets of langerhans causing destruction of islet cells and producing diabetes. Compounds such as dithizone, EDTA, 8-hydroxy quinoline are used to induce spontaneous type 2 diabetes in experimental animals.

Dithizone at dose levels of 40-100mg/ kg (i.v) produces type two diabetes in mice, cats, rabbits and golden hamsters.

Procedure

- Divide Adult rabbits weighing 1.8-2 kg into two groups of six animals each.
- Weigh an exactly amount of Dithizone and dissolve in dilute ammonia solution (0.2-0.5%)
- Warm the solution is to 60-70 C for 10 minutes to aid solubility of dithizone.
- Inject Dithizone at a dose level of 50-200 mg/ kg, produce triphasic glycemic reaction.
- Observe initial hyperglycemia after 2h & normoglycemia after 8h, which persist for up to 24h, permanent hyperglycemia after 24-72 h.
- Administer the drug sample to be analysed through a suitable route and determine blood glucose.

Models For Insulin Sensitivity And Insulin Like Activity[3,6,7,10,18,19,20-23]

1. Euglycemic clamp technique

Principle

This method has proved to be a useful technique of quantifying *in vivo* insulin sensitivity. A variable glucose infusion is delivered to maintain euglycemia during insulin infusion. The net glucose uptake is quantified and sensitivity of body tissue to insulin determined

Procedure

- Adult albino rats weighing 150-200 g are fasted overnight and anaesthetised with pentobarbital (40mg/ kg i.p).
- Insert Catheters into jugular vein and in femoral vein for blood collection & insulin and glucose infusion respectively.
- To evaluate insulin action under physiological hyperinsulinaemia (steady state plasma insulin concentration during the clamp test is around 100 U/ dl) and maximal hyperinsulinaemia, use two insulin infusion rates i.e. 6 and 30 U/kg/min.
- Determine the blood glucose concentrations from samples collected at 5 min intervals during the 90 min clamp test. Adjust the glucose infusion rate so as to maintain the basal levels.
- Calculate the glucose metabolic clearance by dividing the glucose infusion rate by steady state blood glucose concentration.
- Administer the drug sample to be analysed through a suitable route and determine the blood glucose.

Some of the Indian medicinal plants with antidiabetic properties are listed below-

Plant Name	Ayurvedic/common name/herbal formulation
Annona squamosa	Sugar apple
Artemisia pallens	Davana
Areca catechu	Supari
Beta vulgaris	Chukkander
Boerhavia diffusa	Punarnava
Bombax ceiba	Semul
Butea monospermas	Palasa
Camellia sinensis	Tea
Capparis deciduas	Karir or Pinju
Caesalpinia bonducella	Sagarghota, Fevernut
Coccinia indica	Bimb or Kanturi
Emblica officinalis	Amla, Dhatriphala, a constituent of herbal formulation,"Triphala"PitangakrimihritaBurGudmar or MerasingiAnantamul
Eugenia uniflora	Pitanga
Enicostema littorale	Krimihrita
Ficus bengalenesis	Bur
Gymnema sylvestre	Gudmar or MerasingiAnantamul
Hemidesmus indicus	Anantamul
Hibiscus rosa-sinesis	Gudhal or Jasson
Ipomoea batatas	Sakkargand
Momordica cymbalaria	Kadavanchi
Murraya koenigii	Curry patta
Musa sapientum	Banana
Phaseolus vulgaris	Hulga,white kidney Bean
Scoparia dulcis	*Sweet broomweed*
Vinca rosea	Sadabahar
Withania somnifera	*Ashwagandha*

DISCUSSION

The above mentioned models have given broad spectrum for the evaluation of the anti-diabetic activity, each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying aetiopathogenic mechanisms that could also operate during the evolution of type 2 diabetes in humans. Hence, care must be taken in interpretation and extrapolation of the results obtained from these animal models to humans. In the screening programme of anti-diabetic compounds, it is particularly important to note that some animal models are better suited to screen particular class of anti-diabetic compounds [2,3,5,17,21]. Since initial medicinal chemistry campaigns and screening, generally require the testing of many compounds in the industrial research environment, use of smaller animal models such as mice, will reduce the expense of producing test materials while some advanced efficacy studies or toxicological examinations which require invasive procedures and large blood and tissue samples, may be facilitated by using animals with large body size such as rat or other non rodents. Further, the selection of particular animal model is particularly depending on the investigator's choice particular strain, aim of scientific strategy, type of drug being sought, institutional financial and facility resources in the type 2 diabetes research and pharmaceutical drug discovery and development programme.

REFERENCES

1. Hakim ZS. Potential Antidiabetic Agents from Plant Sources, Pharmacological Aspects. *Indian J Natural Product*, 111, 1995, 3.
2. Turner R. Screening Method in Pharmacology Anti-diabetic agent, Academic Press New York, London, 13, 1965, 158.
3. Gupta SK. Drug Screening Methods, Anshan publication, New Delhi, 2006, 254.
4. Bell RH. Animal models of diabetes mellitus, physiology and pathology. *J Surg Res*, 35, 1983, 433-60.
5. Grau U. Stable insulin preparation for implanted insulin pumps, Laboratory and animal trials. *Diabetes*, 36, 1987, 1453-1459.
6. Kumar S. Animal models in diabetes mellitus. *Indian J Exp Biol*, 35, 1997, 1141-5.
7. Parmar NS. Screening Methods in Pharmacology, lavoisier publication, New Delhi, 2006 524.
8. Shafrir E. Animal models of non-insulin dependent diabetes. *Diabetes Metab Rev* 1992, 8 , 179-208
9. Pederson RA. Non insulin dependent animal models of diabetes mellitus, Experimental models of diabetes Florida, USA, CRC Press LLC, 1999, 337-98
10. Reed MJ. In vivo and in vitro models of type 2 diabetes in pharmaceutical drug discovery. *Diabetes Obes Metab*, 1, 1999, 75-86
11. Vogel HG. Drug discovery and evaluation, Pharmacological assays Heidelberg, Berlin, Springer- Verlag, 1997.
12. Agarwal SS. Herbal Drug Technology,Universities press ndia Ltd, Hyderabad, 2007, 525.
13. Srinivasan K. Animal models in type 2 diabetes research, an overview. *Indian J Med Res*, 125, 2007, 451-472.
14. Frederick E. Ethical Issues Involved in the Development of Animal Models for Type I Diabetes. *ILAR Journal*, 351, 1993.
15. Weksler S. The newly inbred Cohen diabetic rat a non obese normolipidemic genetic model of diet induced type 2 diabetes expressing sex differences. *Diabetes*, 50, 2001, 2521-9.
16. ADA. American Diabetes Association Responsible use of animals in research. *Diabetes*, 34, 1985, 714.
17. Barker CF. Animal models of diabetes and immunological problems with islet allografts. *Trans Am Soc Artif Intern Organs*, 28, 1982, 691-699.
18. Gelati KN, Diabetic relinopathy in Spontaneous Animal Models of Human Diseases, vol I, New York, Academic Press 1979, 177-178.
19. Yanagawa T. Effect of antidiabetic medications on micro albuminuria in patients with type 2 diabetes. *Metabolism*, 53(3), 353-357.
20. Babu V. Anti-hyperglycemic activity of *Cassia kleinii* leaf extract in glucose fed normal rats and alloxan-induced diabetic rats. *Indian J Pharmacol*, 34, 2002, 409-15.
21. Yoshioka M. A novel locus, Mody4, distal to D7 Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 Akita mutant mice. *Diabetes*, 46, 1997, 887-94.
22. Winzell MS. The high-fat diet-fed mouse, a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. *Diabetes*, 2004, 53(3), S215-9.
23. Mukherjee PK. Hypoglyca Mukherjee PK. Hypoglycaemic activity of Cassia fistula Linn Leguminosae Leaf in Alloxan induced Diabetic Rats. *Ethanobotany india*, 1997, 9, 35-38.
24. Sarr M. Pancreas in Experimental Surgery and Physiology Induced Animal Models of Human Disease, Baltimore, Md, Williams & Wilkins, 1988, 204-21.