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A NOVEL PROTOCOL DESIGN TO EVALUATE THE PREVENTIVE/ PROPHYLACTIC PROPERTY OF DRUGS/ PLANT PRODUCTS/ NEUTRACEUTICALS AGAINST EXPERIMENTALLY INDUCED IABETES IN ANIMAL MODELS.

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ABSTRACT

Objectives

To establish a novel methodology for the evaluation of prophylactic property of drugs/ plant products against experimentally induced diabetes in animal models.

Materials and methods

Test drug is administered by oral/ i.v. route to animals every day for 90 days. On 91st day, diabetes is induced by chemical method. Test drug administration is continued for another 4 days i.e. from 91st to 94th day.

CBG, lipid profile, body weight are measured once in 15 days till 90 days and everyday for next 10 days by any standard method. After 3rd day of diabetes induction, test rats are not given any drugs, but regular feed is continued upto 10th day.

Results can be analysed as follows

CBG is recorded on 2nd/3rd day after diabetes induction and analysed as:

If average CBG/ lipid levels in the test group is :-

i)Same or more compared to the diabetic control group: infers that there is no prophylactic activity of the evaluating product.

ii) Less than the diabetic control group : infers that the test evaluating product has antihyperglycemic activity indicating the possibility of prophylactic property.

ii) Less than the non-diabetic control group at any time of the experiment(1st to 94th day): infers that the evaluating product has a positive tendency of hypoglycemia with possibility of treatment property.

Conclusion of proposed study

This novel methodology of investigating a drug for its prophylactic property against chemically induced diabetes in animal models may be useful to evaluate a product with future perspective targeting preventive approach in prediabetics or high risk individuals of type 2 diabetes and normal individuals . This novel approach may be useful to screen drugs / plant products / neutraceuticals likely to postpone onset of diabetes or development of its complications.

KEY WORDS: prophylactic, animal diabetic model, antihyperglycemic, chemical method.

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INTRODUCTION

Diabetes Mellitus (DM) is one of the most common endocrine diseases in the world characterized by the state of hyperglycemia caused by defect in insulin secretion or insulin action or both.[1] Unfortunately, DM in the younger age group has been on the rise[2,3] and there is an urgent need to combat this metabolic disorder in this age group of people because the duration of diabetes is more in this group. This group of patients are prone for some long term complications like nephropathy, neuropathy, retinopathy early in their life. The global prevalence of diabetes is estimated to increase from4% in 1995 to 5.4% by 2025[4]. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by 2025[5]. WHO has predicted that the major burden will occur in developing countries. Studies conducted in India have highlighted that not only is the prevalence of diabetes high but also that it is increasing rapidly in urban population particularly in younger age group[4]. It is estimated that there are approximately 33 million adults with Diabetes in India. This number is likely to increase to 57.2 million by the year 2025[4]. The long asymptomatic phase of type II DM gives a general misconception that it is a mild disease but leads to a grim scenario where 15-20% of Patients present with micro or macrovascular complications at the time of diagnosis. The dreaded microvascular complications of DM are related to the degree of metabolic decompensation and the same in high risk individuals can be prevented or postponed by not only achieving persistant and control[6] but also by sensitising the individuals for prophylactic therapy in prediabetics or high risk individuals.

By the time a patient is diagnosed with type II DM, 70% of β-cells are lost[2] whereas in IFG(FBS 100-125mg%,PPBS< 140mg%)[2,5], IGT(FBS<100mg%, PPBS 140-200mg%)[2,5]the loss is of a lesser degree. Hence, preservation of remaining β-cells and enhancing insulin induced signals of glucose entry to peripheral cells (GLUT-4 transcription) is very important in prediabetics(IGT and IFG)[2]. This can be achieved with the group of drugs having prophylactic property with either one or both of the above actions. Although uncontrolled hyperglycemia is one of the most important causes of early development of complications in diabetes, the total duration of diabetes[2] in any patient is also an important factor contributing to the development of complications. Hence young diabetics are prone for complications at an early age as the duration of type II diabetes will be more than in maturity onset type II DM. An ideal treatment for diabetes would be a drug that not only controls the glycemic level but also prevents the development of complications of diabetes[7]. Although there are effective drugs for therapy of diabetes and to prevent its complications, the drug therapy for postponement of development of diabetes in prediabetics and high risk individuals is effectively not available. Troglitazone[5], Metformin[5] and acarbose[5] can sometimes be used in prediabetics and selective high risk individuals like hypertensive heart disease, dyslipidemia, alcoholics, gestational DM, obesity, those with family history of diabetes and coronary artery disease[2] who may be suggested to undergo prophylactic therapy to prevent or postpone the development of diabetes with the help of drugs or plant products or neutraceuticals having prophylactic property.

With this scenario in mind, there is a definite need for effective prevention or postponing of manifestations of DM and to delay or prevent the onset of complications by drugs or plant products or neutraceuticals. Inspite of availability of many animal models as screening methods for the evaluation of therapeutic property of drugs, there is very little animal experimental methodology available for evaluating prophylactic property of drugs. Hence there is a definite need for animal experimental screening methodology for evaluation of preventive or prophylactic property of drugs or plant products or neutraceuticals in experimentally induced hyperglycemia and dyslipidemia. With this background, an attempt has been made in the present proposed concept to come up with a novel animal experimental methodology for the evaluation of prophylactic property of

drugs or plant products or neutraceuticals supported with scientific background. This experimental methodology in animals can be targeted for future application in human beings not only associated with prediabetic status but also in high risk and normal individuals for prevention of Diabetes mellitus.

MATERIALS AND METHODS

STUDY GROUP OF ANIMALS

Healthy Albino rats of Wistar strain weighing around 200-250 g are selected. They are fed with standard pellet diet and water ad libitum and maintained under controlled environmental conditions. The norms of good laboratory practice must be followed for the care of laboratory animals. The animals are divided into 3 groups: non diabetic control, diabetic control and test groups. The fourth group as standard can be incorporated with pioglitazone or metformin as standard drug.

DRUGS AND CHEMICALS USED

Drug under evaluation
Diabetes inducing chemical agent(alloxan or streptozotocin)
Standard drug- if standard group is selected
Benedict's solution- for measuring urine sugar

OTHER MATERIALS USED

Glucometer with glucose strips
Lipid kit analysing lipid values
Standard dry pellet diet
Metabolic cage
Small animal weighing machine etc.,

EXPERIMENTAL METHODS

Diabetes in animals can be induced by any chemically inducing methods using Streptozotocin[8] or alloxan[9]

PROPOSED METHOD OF INDUCTION OF DIABETES IN RATS

STREPTOZOTOCIN INDUCED DIABETIC MODEL

It is one of the common methods followed. Diabetes can be induced by i.p. injection of STZ (60mg/kg)[8] dissolved in sodium citrate buffer solution in right lower quadrant of abdomen after an overnight fasting in Test & Diabetic control group of rats. An equal volume of buffer is injected into non-diabetic control rats.

METHODOLOGY

The drug under evaluation is administered everyday to the test group animals for 90 days by any acceptable route, oral or i.v.(i.p. can be avoided because of long term administration) & non diabetic control & diabetic control group must be administered with equal quantity of placebo (gum acacia / water) During this period CBG levels[10], blood lipid levels, urine sugar and body weight are measured once in 15 days. On 91st day diabetes is induced in all the groups of rats except in non-diabetic control group by i.p. injection of STZ. After STZ injection, the rat feeding is continued with administration of the test drug for the test group, placebo to Non diabetic control & Diabetic control group of rats (and standard drug to the standard group of animals – if 4th group is incorporated in the Study) for next 4 days also(i,.e. from 91st to 94th day)(the time taken for development of hyperglycemia after chemical induction of diabetes. The CBG, lipid profile, urine sugar, body weight will be measured once in 15 days till 90 days and everyday thereafter for next 10 days (totalling to 100 days) with the help of glucometer and lipid analysing kits.

STATISTICAL ANALYSIS

By any standard method for the evaluation of test drug.

RESULTS CAN BE ANALYSED AS FOLLOWS

The results can be estimated by measuring CBG levels(mg/dl)/ lipid levels(mg/dl)/ urine sugar(by Benedicts test)/ body weight(in gram) in various groups of animals and analysed as below:

i) If the average CBG/ lipid profile levels in test group is same or more compared to diabetic control group of rats---> it gives an inference that there is no prophylactic or preventive activity of the product under evaluation.

ii) If the average CBG / lipid profile levels in test group is less than in diabetic control group---> gives an inference that the drug

under evaluation has antihyperglycemic activity indicating a possibility of preventive or prophylactic property.

iii) If the average CBG / lipid profile levels in test group is less than non-diabetic control at any time of the experiment(1st to 94th day)----> it gives an inference that the drug under evaluation has hypoglycemic activity concluding positive tendancy of hypoglycaemia with a possibility of treatment property.

DISCUSSION

The evaluation of a novel drug with potential prophylactic activity against development of diabetes necessitates selection of proper animal models. The available animal experimental models are targeted mainly on evaluating the drugs which are administered along with the agents inducing hyperglycemia / dyslipidemia but not much studies or methodology is available for evaluation of drugs or plant products administered for a considerably long period of time prior to inducing hyperglycemia to check their prophylactic property. The aim of the present proposed concept is to highlight the detailed methodology of experimental animal models for evaluation of prophylactic / preventive property of drugs or plant products or neutraceuticals against experimentally induced diabetes in animal models.

Table 1

Table showing various parameters on different days in different groups of animals.

	Blood glucose levels(mg/dl)(CBG method) / lipid profile(mg/dl) (lipid kit)/ Urine sugar(mg/dl) / body weight (g)																
	DIABETES INDUCTION ⇩																
Groups	Before induction (DAYS)							After induction (DAYS)									
	0	15	30	45	60	75	90	91	92	93	94	95	96	97	98	99	100
CONTROL																	
DIABETIC CONTROL																	
STANDARD																	
TEST GROUP																	

← DRUG ADMINISTRATION DURATION →------>

CONCLUSION

This proposed protocol design will provide useful knowledge of animal experimental methodology which may help to predict the prophylactic or preventive activity of drugs or plant products or neutraceuticals and identify the new possible approaches to treat prediabetics or high risk individuals and if possible in normal individuals also with a scientific background. This animal methodology may be of better help even before the products are subjected to detailed cellular/ genetic/ metabolic activity and assessment of the particular product.

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