

Methodology of Study of an Antihyperglycemic Traditional Cure with Oral Glucose Tolerance Test to Wistar Rat

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Abstract: Our aim was to expose a method of study an antihyperglycemic traditional cure with oral glucose tolerance test to wistar rat. This methodology began by obtaining plants species component the cure, their extraction and identification of assets. The traditional cure is composited of leaves of both *Heliotropium indicum* (Boraginaceae), *Ocimum gratissimum* (Lamiaceae) and barks of both *Sclerocarya birrea*, (Anacardiaceae), *Khaya senegalensis* (Meliaceae). After, experimental animal is chosen and the blood samples are made. The pharmacological criteria took into account the oral antidiabetic therapeutic as the glibenclamide. Then the glucose overload is absorbed by the animals after administration of the test cure in single or multiple outlets. The anhydrous glucose used to induce experimental diabetes is administered at a dose of 4 g/kg/vo. Finally, we carried out the blood glucose. The methodology of study of an antihyperglycemic traditional cure took account the protection of laboratory animals after experimentation.

Keywords: Methodology, Traditional Cure, Oral Glucose, Wistar Rat

1. Introduction

Experimental diabetes models clearly show that the disease has complex and diverse pathological features. However, each model allows to study a particular aspect, either at the cellular, molecular, biochemical or genetic, with reference to what is observed in humans. The development of these models has enabled a better understanding of the pathophysiology and progression of diabetes, particularly type 2 diabetes, with the aim of antidiabetic therapy.

Methods of study of antidiabetic drugs are many according to the means.

Animal models in vivo and in vitro models are used in research antihyperglycemic agents involved in the pathophysiology of diabetes [16]

In vitro animal models concern isolated organs, used by the in vitro experimental model are the pancreas, liver and muscle.

In vivo models of diabetes are interested in animal models induced by chemicals that are alloxan and streptozotocin to induce animal type 1 diabetes.

Our aim is to expose a method of study an antihyperglycemic traditional cure with oral glucose tolerance test to wistar rat. The traditional cure is composited of leaves of both *Heliotropium indicum* (Boraginaceae), *Ocimum gratissimum* (Lamiaceae) and barks of both *Sclerocarya birrea* (Anacardiaceae), *Khaya senegalensis* (Meliaceae).

2. Methodology and Materials

2.1. Obtaining Plant Species Component Traditional Cure

Heliotropium indicum and *Ocimum gratissimum* grow in

any season throughout the West African sub- region and tropical flowering may extend throughout the year. These two plant species are herbs from about 30 to 80 cm so easy to harvest in vegetation for their medicinal uses [1, 6]. Concerning, *Sclerocarya birrea* and *Khaya senegalensis* stems barks, they are usually available at no out of stock on the major urban markets. Also good preparation and conservation of plant material required washing with distilled water and the correct drying in the open air. All these operations have prevented the degradation of plant material [4].

2.2. Extraction and Identification of Assets

The different amounts of drugs (250 and 50 g) used in the extractions were used to assess their yields extractions and especially to realize the phytochemical sorting. We extracted the active elements of the plant by vacuum evaporation with a Rotavapor at 50°C. This method allowed extraction followed by lyophilization has the merit to dry the extract. It is the most appropriate in the presence of an aqueous solution [11].

The methods of Nemlin [12] and Konkon [7] for the identification of the majority of large chemical groups in drug substances of plant origin.

2.3. Feedings and Blood Samples of the Animal

The Wistar rat called *Rattus norvegicus* is marketed in the world at low cost. In addition, a good prolificacy with a small number of up to 12 has kidded, easy maneuverability [3] has motivated the choice of the rat for the in vivo testing of blood glucose. The volumes of the solutions recommended for feeding rats 1ml/100g body weight were absorbed by the animals without effect on [10] lungs. Indeed, an enter of gavages solution into that body immediately causes the death of the animal.

The unique extract administration that preceded the repeated dose in free days allowed reduces fatigue related to animal feedings. In addition, during the glucose testing, alternating single and repeated doses was adopted in particular with the traditional preparation performed on 32 rats with the aim of halving the number of animals as recommended bioethics [14].

Blood samples for glucose values require of rehearsals that could affect the total blood volume of the rat. For this reason, the drop of blood which is a lower amount to the volume of blood recommended was 0.06% of body weight of the animal per day [3] allowed the realization of samples without causing fatigue and suffering in animals. The ether anesthesia in a glass bell ended the pain of animals [14].

The blood collection time can reach 5, 10 and 12 hours [13] after administration of the test product. The second case (10 hours) was adopted in our experiment because the single dose samples were taken between 7 and 18 hours with a frequency of one sample every two hours. Thus, the five blood samples were allowed to follow the daily changes in blood glucose, which is not possible with the sampling method in the orbital sinus of the animals. In the latter, scarring can last a week.

2.4. Pharmacological Criteria

Oral antidiabetic drugs available in the market are two folds [8]:

First biguanides that may lower blood glucose levels in hyperglycemic subject without noticeable effect in a normoglycemic subject, then the sulfonylureas, which act by reducing blood glucose levels throughout even if the subject is fasting hence the term hypoglycemic. These two types of activities identified in the various specialties marketed, so we have imposed a dual study including fasting hyperglycemia and after considerable hyperglycemia situation. Thus, glibenclamide is used as a reference and used oral antidiabetic therapeutic dose of 0.2 mg/kg/vo in experimental studies [10, 15].

2.5. Induction of Hyperglycemia

Hyperglycemia is caused by any substance containing a sufficient amount of glucose absorbed and constitutes an experimental model to evaluate the antihyperglycemic activity of medicinal substances of plant origin [5, 11]. The administration of glibenclamide or traditional cure 1 hour before administering the glucose overload (4 g/kg), allowed to match the moment of maximum activity related to the hyperglycemic glucose overload with the maximum of antihyperglycemic activity of the glibenclamide (0,2 mg/kg) [5, 10].

2.6. Dosing Blood Glucose

The use of glucose meter reader to dose blood glucose [10, 13] including «One Touch», it can be performed at any time of the day, regardless of the location and the number of times that you want. The availability and accessibility added to the previous quality make the meter a better way to perform different dosages of blood glucose study [11].

The method of sampling drops of blood for blood glucose is adapted to a glycemic drive because it would limit the suffering of animals. Better, it make the healing easy of animals in less than a week instead of several months in other types of samples [11].

Prior to the determination of fasting blood glucose and after regular feeding rats helped determine respectively the average values fasting and those after normal diet. The evaluation of the antihyperglycemic traditional cure improved on blood sugar 32 male rats in accordance with the principle of bioethics applicant a reduction in the number of animals in biological tests [15].

The test performed antidiabetic activity has taken place as compared to control rat. They have received distilled water because the influence of this solution on blood glucose is negligible. Blood glucose observed in this batch was therefore reflecting the usual progression in all rats fasted from the 2nd to the 10th time corresponding to the period of the tests.

Thus, based on Student statistical tests to perform comparisons «test rat – control rat», we wanted at first whether it is a natural evolution of hyperglycemia per os. In this case the observed difference between the average (test rat

– control rat) is not significant ($n = 8$; $p > 0.05$) statistically. Secondly, when the tested product has effects on blood glucose; in this case the variations observed were significant ($n = 8$; $p < 0.05$).

3. Result and Practical Data of Oral Glucose Tolerance Test

Two case studies of oral glucose tolerance in rats [2, 14] are showed to analyze. In the present investigation, 36

normal rats were fasted for 12h and assigned randomly into 6 equal groups ($n = 6/\text{group}$) [14]. They were fed orally at with aqueous bark extract of *Strychnos henningsii* (Loganiaceae) at the doses of 125, 250, 500 mg/kg and glibenclamide (0,6mg/kg) using gavages. The remaining groups consisting of normal and diabetic rats were treated orally with the distilled water. Glucose (2g/kg) was orally administered 30min prior to the extract administration and blood was withdrawn from the tail vein at 30, 60 and 90min.

Table 1. Effect of oral administration of extract aqueous of *Strychnos henningsii* on oral glucose test in male Wistar rats [14].

Treatments	0 minute	30 minutes	60 minutes	90 minutes
Normal control	4,6 ± 0,4	4,90 ± 0,53	4,4 ± 0,3	4,13 ± 0,32
Diabetic control	14,73 ± 8	18,63 ± 7	21,64 ± 7,2	20,64 ± 5,10
SH 125 mg/Kg	5,50 ± 1,6	10,4 ± 1,59	18,6 ± 2,42	15,77 ± 2,84
SH 250 mg/Kg	5,14 ± 2,20	6,87 ± 2,3	10,20 ± 2,4	7,93 ± 1,93
SH 500 mg/Kg	5,47 ± 2	7,10 ± 0,92	7,5 ± 1,98	7,53 ± 1,98
Glibenclamide (0,6 mg/Kg)	4,40 ± 2,5	5,03 ± 1,59	4,072 ± 4,2	4,6 ± 2,84

Values are mean ± SD of 6 rats in each group. Tests values carrying superscripts different from the control for each parameter are significantly different ($p < 0,05$).

Different in blood glucose of each group other final-initial values: Diabetic 15, 31 mmol; 125 mg/Kg (↓2,83 mmol/L); 250 mg/Kg (↓2,83 mmol/L); 500 mg/Kg (↑10,3 mmol/L). Glibenclamide suppressed increase blood glucose near normal. Where ↓mean increase and ↑ represents decrease.

In the other investigation, a total 20 normal rats were taken and divided into four groups for oral glucose tolerance test (each group 5 rats) with an antihyperglycemic plant, *Cynoglossum zeylanicum* (Boraginaceae) [2]. The

concentrated ethanol extract of this medicinal plant was used for the oral glucose tolerance test. After 60 minutes of drug administration, the rats were orally treated with 2g/kg of glucose. The blood samples were collected through femoral vein at 0, 30, 60, 90, 120 minutes. Blood glucose level was estimated at various time intervals.

The different treatments are divided:

Group I - Normal control received 0.9% saline; Group II - Ethanol extract of *Cynoglossum zeylanicum* whole plant (100 mg/kg p.o); Group III - Ethanol extract of *Cynoglossum zeylanicum* whole plant (150 mg/kg p.o); Group IV - Standard drug glibenclamide (600 µg/kg p.o)

Table 2. Oral glucose tolerance test rats after treatment with *Cynoglossum zeylanicum* (Boraginaceae) whole plant extract [2].

Blood glucose level (mg/dl)		0 minute	30 minutes	60 minutes	90 minutes	120 minutes
Treatments	Doses					
Group I	0.9%Saline	91.85±2.88	134.72±3.83*	152.31±3.29*	121.42±2.19*	82.53±1.53
Group II	100mg/kg	84.62±1.54	103.14±2.39	123.91±2.63*	92.17±1.72	69.23±1.02
Group III	150mg/kg	76.29±1.35	118.28±1.66	137.32±2.39*	109.64±1.54	78.27±1.14
Group IV	600µg/kg	73.72±1.22	109.48±1.54	128.37±1.07*	102.98±1.04	72.53±1.11

Values are mean ± SEM (N=5):*P<0.05, statistically compared to 0 min to other respective groups.

The blood sugar levels of *Cynoglossum zeylanicum* (100 and 150 mg/kg) treated groups were found compared to be control groups (groups I et IV) and the effects were dose-dependent. Group III and IV glucose lowering efficiency between 90-120 minutes.

4. Discussion

The different models of study the antidiabetic plants are existed. Our concern is to find a model for studying the antidiabetic activity of the drug that preserves the animals tested after the experiments. Among them, the oral glucose tolerance test steps we explained in this study permitted to identify the antihyperglycemic plants by glucose induction to 2 at 4 g/kg/vo in rat Wistar weighing 110 g at 255 g [2, 11, 14]. The experimentation of antihyperglycemic activity in the oral glucose tolerance test to the traditional cure,

Cynoglossum zeylanicum and *strychnos henningsii* were respectively [2, 11, 14].

This important reduction of blood glucose were encouraging compared those of the oral reference antidiabetic like the glibenclamide (0,2 mg/kg at 0,6 mg/kg). Its were respectively after oral administration of plant extract and glibenclamide 42%/38% [11], 17%/20% [14] and 25,8%/20% [2] with the doses 35 mg/kg,125 mg/kg,150 mg/kg. So, the containing result of these three studies demonstrated that the oral glucose tolerance test can valid to evidence the antihyperglycemic activity of certain medicinal drug.

In our study, a concentration of anhydrous glucose was 4 g/kg per os was used to induce franc hyperglycemia in Wistar rats. Thirty minutes after glucose administration, the mean of blood glucose peak as 245 ± 11 mg/dl appeared [10, 11]. The way of obtaining a franc hyperglycemia to test our traditional cure is confirmed by other studies on Wistar rats. Indeed, by

administration of the same 4g/kg anhydrous glucose per os the other author [13] found that the blood glucose peaks were 225 ± 17 mg/dl.

In addition, the value of the average blood glucose peak in our study is higher than the normal value (108.6 mg/kg) obtained after proper feeding of farmed Wistar rats [11]. In another experiments, hyperglycemia respectively of 170 mg/dl in rabbits [5], 18,6mmol/l [14], and 152,31 mg/dl [2], both in normal rats were sufficient to study the antihyperglycemic activity an induction of 2 g/kg glucose.

Better, the induction of hyperglycemia oral glucose load that is the model used in our experience (traditional cure) and to *Cynoglossum zeylanicum* (Boraginaceae) aqueous extract helped to preserve the treated rats [10,11]. Also, in the oral glucose tolerance test, the integrity of the experimental animal was preserve like recommendation [15] in the methodological general principles for research and evaluation on traditional medicine.

In fact, the animal models made diabetic with streptozotocin or alloxan had generally the pancreatic cells destroyed and irreversibly [9]. This may be an animal ethical problem in tests using laboratory animals.

To remedy this, we proposed for antidiabetic drug study, Biobreeding rat. This laboratory animal is an in bred strain that spontaneously develops autoimmune type 1 diabetes. Like NOD mice, Biobreeding rats are used as an animal model for type 1 diabetes. The strain re-capitulates many of the features of human type 1 diabetes, could contributed greatly to this kind of research.

5. Conclusion

The methodology used in this study includes several successive stages to study the antihyperglycemic activity of a traditional oral drug in Wistar rats. Those are obtaining plant species component traditional cure, extraction and identification of assets, feedings and blood samples of the animal, pharmacological criteria, induction of hyperglycemia, dosing blood glucose and practical data of oral glucose tolerance test. In this methodology, laboratory animal was preserve after experimentation, so it could be repeated greatly. And a pharmacovigilance study would be considered with this traditional antihyperglycemic drug for clinical trials.

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References

- [1] Aké AL. Abrégés de médecine et pharmacopée ivoiriennes: quelques plantes employées, dans les soins de santé primaire. Editions NEI-CEDA. Abidjan, 2012, 86-94.

- [2] Anitha M, Sakthidevi G, Muthukumarasamy S, Mohan VR. Effect of *cynoglossum zeylanicum* (Vahl ex Hornem) Thunb. ex Lehm on oral glucose tolerance in rats. J of Applied pharmaceutical Science, 2012; 2, 11: 75-78.
- [3] Bauck. Basic anatomy, physiology, husbandry and clinical techniques. Part I. In Hillyer, Quesenberry. Ferrets, rabbits and rodents. Clin med and surg, saunders, 2004; 26, 291-297.
- [4] Debuigue G. Larousse des plantes qui guérissent. Librairie Larousse, 2010; p. 5-6.
- [5] Keita A, Mariko E, Haidara TK. Etude de l'activité hypoglycémiant des feuilles de *Sclerocarya birrea* (A. Rich) Hochst. (Anacardiaceae) Pharm. Méd. Trad. Afr. 1998, 10: 16-25.
- [6] Kerharo J, Adam JC. La pharmacopée sénégalaise traditionnelle: plantes médicinales et tropicales. Paris, Ed Vigot et frères, 1974; 433-35.
- [7] Konkon NG, Adjoungou AL, Manda P. Toxicological and phytochemical screening study of *Mitragyna inermis* (Rubiaceae). Antidiabetic plant. J of medecinal plants research, 2008; 2, 10: 279-284.
- [8] Lokrou A. Guide de prise en charge des diabétiques. Collection santé, Editions Universitaires de Côte d'Ivoire, 2008; 212p.
- [9] Mohamed A, Tanko Y, Okasha MA. Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozotocin-induced diabetic Wistar rats. Afr J Biotechnolog, 2007; 6, 18: 2087-90.
- [10] Monteomo GF, Tehoua L, Ouattara H et al. Comparative antihyperglycemic activity of *Sclerocarya birrea*, *Khaya senegalensis*, *Heliotropium indicum* and *Ocimum gratissimum* to rats Wistar. Am J Biosciences, 2014; 2, 2: 60-63.
- [11] Monteomo GF. Valorisation de la pharmacopée africaine: étude de l'activité antihyperglycémique d'une préparation traditionnelle améliorée composée de *sclerocarya birrea* (Anacardiaceae), *Khaya senegalensis* (Meliacée), *Heliotropium indicum* (Borraginacée) et *Ocimum gratissimum* (Lamiacée). Doctorat des Universités, Option, Biologie Humaine, Université FHB-Abidjan, UFR-SM, 2015; N° 26, 172p.
- [12] Nemlin J, Brunel JF. Travaux pratiques de matière médicale, 3^{ème} année, Paris, Masson, 1995-1996: 39-43.
- [13] Ojewole JAO. Laboratory evaluation of the hypoglycemic effect of *anarcadium occidentale* Linn. (Anarcadiaceae) stem-bark extracts in rats. Methods and findings in experimental and clinical, 2003; 25, 3: 199-204.
- [14] Oyedemi S, Bradley G, Afolayan A. Antidiabetic activities of aqueous stem bark extract of *Strychnos henningsii* Gild in streptozotocin-nicotinamide types 2 diabetic rats. J of Pharmaceutical Research, 2012; 11, 1: 221-228.
- [15] Vilmer JB. Ethique animale. Que sais-je. Presses universitaires de France, Paris, 2015; 79-90p.
- [16] Zhao VW, James MU, Schraw TD. Panic-Attac: A Mouse model for Inducible and Reversible β -Cell Ablation. Diab. 2008; 57, 8: 2137-2148.