www.ijrps.pharmascope.org

ISSN: 0975-7538
Research Article

Evidence based analysis of chemical method of induction of diabetes mellitus in experimental rats

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ABSTRACT

There have been several reports expressing doubt concerning the use of chemical induction of diabetes mellitus in experimental animals as a suitable model for studying type II diabetes mellitus. This study is designed to assess whether there is a significant difference in response by chemically (alloxan) induced hyperglycaemic (AIH) and oral glucose loading induced hyperglycaemic (GIH) wistar rats to antidiabetic agents. AIH was achieved through intraperitoneal administration of alloxan monohydrate (150mg/kg body weight) to rats, while GIH was induced in another group of rats by administering orally (2.5g/kg body weight) of glucose solution. The two sets of rats represent hyperglycaemia with insufficient pancreatic activity and hyperglycaemia with intact pancreas functions respectively. Two standard antidiabetic agents namely: metformin(20mg/kg) and tolbutamide(20mg/kg body weight) and two plant extracts namely: *Vernonia amygdalina* and *Mangifera indica* (200mg/kg body weight) leaves extracts were used as the antidiabetic agents. The results showed that, administration of 150mg/kg of alloxan monohydrate successfully raised the blood glucose levels in 75% of the treated rat's to ≥ 150mg/dl after 72 hours, while oral loading of the rats with 2.5g/kg body weight of glucose solution produced hyperglycemia in 70% of the rats. The maximum blood glucose levels reached after induction with glucose and alloxan were 338.3±61.2mg/dl and 514.7±34.7mg/dl respectively. The highest percentage reductions in blood glucose level achieved after the treatment with the agents were 54.3% in GIH and 67.1% in AIH. The Spearman rank correlation (Rs) calculated for the two sets of data was 0.8261 which is very significant. This finding showed that, chemical induction of diabetes mellitus has no differential effect on the response of antidiabetic agents and the method is suitable for studying type II diabetes mellitus in experimental animals.

Keywords: Alloxan monohydrate; diabetes mellitus; experimental rats; glucose.

INTRODUCTION

Experimental diabetes mellitus has been induced in laboratory animals by several methods that include: chemical, surgical and genetic (immunological) manipulations. Most experiment in diabetes is carried out in rodents, although some studies are still performed in larger animals (Rees and Alcolado 2005; Urban et al., 2008).

The majority of studies published in the field of ethnopharmacology between 1996 and 2006 employed chemical induced model. Streptozotocin (STZ, 69% and alloxan (31%) are by far the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease (Rydgren et al., 2007). Both drugs exert their diabetogenic action when they are administered parenterally (intravenously, intraperitoneally or subcutaneously). The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status (Balamurugan et al., 2003).

Alloxan, a well- known diabetogenic agent is widely used to induce type 2 diabetes in animals (Viana et al., 2004). The drug and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydroxyl radicals are formed by fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of *beta* cells (Szkudelki, 2001). Thus alloxan induced diabetes mellitus served as a pathological biomodel for testing a substance with supposed antioxidant activities *in vivo* (Bartosikova et al., 2003). One of the targets of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in *beta* cells exposed to alloxan (Takasu et al., 1991). The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent alloxan. With this method Macedo et al. (2001) induced diabetes mellitus in experimental rats.

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Received on: 01-02-2010
Revised on: 23-03-2010
Accepted on: 25-03-2010

Although the use of chemical (alloxan and streptozotocin) to induce type 2 diabetes mellitus is the most widely used method, recently it has been widely criticized as being unsuitable because the chemical seems to destroy the β cells and create more of type 1 than type 2 diabetes (Masiello, 2006). The present study examined the pattern of hyperglycaemia induce by chemical agent (alloxan) and oral glucose loading in experimental rats, and their responses to antidiabetic agents in order to deduce the suitability or otherwise of chemical induction as a model for studying type 2 diabetes mellitus in experimental animals.

MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology, College of Health Sciences (CHS), Usmanu Danfodiyo University, Sokoto (UDUS) between January and June 2009. The study protocol was approved by the Ethics Committee of the Institution.

Animals

Male wistar rats (163.5 ± 24.23 g) were used for the study. They were obtained from the animal stock of the Department of Pharmacology, CHS, UDUS. The rats were kept in rat cages, fed with commercial rat pellets (Pfizer Feeds, Nigeria Ltd.) and allowed free access to fresh water *ad libitum*.

Extract preparation

The fresh leaves of *Vernonia amygdalina* and *Mangifera indica* were collected from source after proper identification. They were washed with tap water to remove the settling dirt and air-dried at room temperature to a constant weight. The dried materials were then comminuted into coarse powder using mortar and pestle. 100g of each powder was extracted in 1000 ml of distilled water for 48 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whitman no. 1 filter paper. The resulting filtrates were oven-dried (Temperature 45^{o}C) and preserved in a deep freezer at about -17^{0}C pending the commencement of the study. The filtrate was later reconstituted separately in distilled water to give the required doses used in this study.

Drug preparation

Metformin (Hovid, Ipoh Malaysia, Batch No.VUDIA 11-0, 500mg) and tolbutamide tablets (Hochest Pharmaceuticals Ltd., 500mg) were purchased from a local pharmaceutical company in Sokoto. The tablets were finely powered and suspended in distilled water at a concentration of 5mg/ml and given at 20mg/kg body weight of the animals.

Animal treatment

In the first phase of this study, 60 male wistar rats were selected for used. The baseline blood glucose level of each of the animal was taken. 150mg/kg (b.wt.) of alloxan monohydrate was administered intraperitoneally to the rats after being deprived of food for 18 hours to induce hyperglycaemia (Yanarday and Colac, 1998). The blood glucose levels in the animals were measured 72hours after the drug administration through tail tipping using Glucometer (Accu-Chek, Active, Roche Diagnostic's 9115 Hague road, Indianopolis, 46256 Lot No 115764) and those found to be diabetic (serum glucose ≥ 150mg/dl) were selected for the study. Twenty five diabetic rats were selected and divided into five groups (n = 5) labeled A, B, C, D and E. The antidiabetic agents [metformin (20mg/kg), tolbutamide (20mg/kg), *Vernonia amygdalina* and *Mangifera indica* (200mg/kg) aqueous leaves extracts] were administered orally to four groups (A – D) and their blood glucose levels measured at 30, 60 and 120 minutes intervals. The fifth group (diabetic control) and the sixth group (normal control) which was made up of non diabetic rats were equivalent volumes of normal saline.

In the second phase, 30 male wistar rats were selected and divided into 6 groups (n=5), labeled 1- 6. Their baseline blood glucose levels were measured. The rats in group 1(normal control) were given normal saline orally. Those in groups 2 – 6 were treated with 2.5g/kg (b.wt) of oral glucose solution to induce hyperglycaemia (Meiton, 2006). After 5 minutes, all the animals in groups 3-6 were treated with the same antidiabetic agents as above and those in group 2 (diabetic control) were left without additional treatment The blood glucose levels of these animals were measured at 30, 60 and 120minutes intervals through tail tipping using a Glucometer (Meiton, 2006) .

Statistical analysis

Data were expressed as mean of five replicates plus or minus standard error of mean. The means were subjected to one way analysis of variance (ANOVA) and results that showed statistical significant differences were further tested using Turkey – Kraimer multiple comparison test. Values were considered statistically significant at $P < 0.05$.

RESULTS

Intraperitoneal administration of 150mg/kg of alloxan monohydrate induced hyperglycaemia (Blood glucose level ≥150mg/dl) in about 75% of the treated rats. This was higher than the 70% produced by oral glucose loading. The peaks blood glucose levels reached in AIH and GIH rats were 514.7±34.7 and 338.3±61.2mg/dl with the means as (376 and 136) mg/dl respectively. The level of blood glucose remained fairly stable at about 250mg/dl in the alloxan diabetic rats throughout the 2 hours observatory period but fluctuated widely between 251 and 338mg/dl in the glucose loading diabetic rats (Table 1& 2). *Vernonia amygdalina* produced the highest percentage reduction (67.1%) of blood glucose in AIH rats while tolbutamide (54.3%) was the highest in the GIH rats.

Table 1: Response by alloxan induced diabetic rats to selected antidiabetic agents

Treatment (mg/kg)	Time intervals (minutes)				
	0	30	60	120	% Reduction
Normal control	112.7±8.0	113.0±8.1	108.0±7.6	107.0±8.1	
Diabetic control	252.4±3.5	257.4±3.8	262.3±2.7	250.2±4.1	
Metformin(20)	489.3±50.9	367.0±12.5	239.0±37.8*	178.3±61.9**	63.6
Tolbutamide(20)	227.2±29.7	194.3±31.7	168.0±45.3	146.3±45.3*	35.6
Vernonia amygdalina(200)	514.7±34.7	356.7±31.7	253.3±51.1	169.3±21.4***	67.1
Mangifera indica	436.3±16.1	389.7±81.8	294.7±53.3	272.7±43.6*	37.5

N=5; * = P > 0.5; ** = P < 0.01; *** = P < 001; % = Percentage.

Table 2: Response by glucose induced diabetic rats to selected antidiabetic agents

Treatment (mg/kg)	Time intervals (minutes)				
	0	30	60	120	% Reduction
Normal control	112.7±7.2	126.3±9.2	111.7±7.6	101.0±2.6	
Diabetic control	117.0±8.0	338.3±61.2	289.3±64.8	262.3±46.9	
Metformin(20)	109.0±5.3	140.0±1.9	127.3±3.5	92.6±5.6*	33.9
Tolbutamide(20)	106.0±7.8	180.0±12.6	124.3±9.5	104.0±2.5***	54.3
Vernonia amygdalina(200)	119.1±5.3	138.3±8.1	116.7±9.5	97.7±5.7*	29.4
Mangifera indica	95.3±5.2	168.7±6.6	133.0±4.8	93.3±3.4**	44.7

N=5; * = P > 0.5; ** = P < 0.01; *** = P < 001; % = Percentage.

DISCUSSION

Alloxan monohydrate has been utilized by several researchers to induce diabetes in experimental animals (Viana et al., 2004; Antia et al., 2005). But the method has often been criticized as being unsuitable for studying type 2 diabetes mellitus because it causes pancreatic β cells destruction thereby creating insulin deficiency and a type I like form of diabetes (Szkudelski). The present study has shown that, alloxan administration produced a higher and sustainable form of hyperglycaemia. This finding agrees with that of Korec (1988) who reported a more pronounced and sustained hyperglycaemia following chemical induction with alloxan when compared to the oral glucose loading in rats.

The treatment of the diabetic rats with selected anitidiabetic agents produced varying degrees of blood glucose reduction. The choice of metformin and tolbutamide was to reflect the two major groups of hypoglycaemic agents (sulphonylureas and biquanides) commonly used (Federiuk et al., 2004). The aqueous leaves extracts of *Vernonia amygdalina* and *Mangifera indica* have been reported to possess antidiabetic properties (Thomas 2000; Aderibigebe and Emudianughe 1999; Erato et al., 2005). Their inclusion in this study was to reflect the current trend of research in this environment which focuses more on medicinal plants and to allow comparison between the orthodox and the unorthodox agents. Metformin and *Vernonia amygdalina* leaves extract produced the most effective blood glucose lowering effect among the AIH rats while tolbutamide and *Mangifera indica* leaves extract was more effective in GIH rats.

Metformin reduces blood glucose level in diabetes mellitus through a non insulin dependent mechanism whereas tolbutamide relies on insulin release from the pancreas to lower the blood glucose (Federiuk et al., 2004). This may be responsible for the variation in the drugs activity among the two groups of animals; one with an intact and the second with a damaged pancreas. The effect of the plant extracts may also follow the same pattern. Although, this suggestion differs from the opinion of Aderibigebe and Emudianughe (1999) who suggested that the antidiabetic activity of aqueous leaves of *Mangifera indica* was due to the reduction of intestinal absorption of glucose.

The Spearman rank correlation (Rs) calculated for the percentage reduction of blood glucose levels in AIH and GIH rats gave 0.8261 which is very significant. This showed that there was no significant difference in the response to the antidiabetic agents by the two groups of diabetic animals. Alloxan administration in experimental animals has been reported to produce pancreatic lesion which is proportional to the dose of the drug administered. And the size of the lesion also correlates with the pancreatic insulin content (McNiell, 1990). This perhaps explains why the drug does not produce absolute but insufficient insulin deficiency in experimental animals. Therefore the experimental dose of the drug must be carefully selected in order to avoid excessive pancreatic tissue damage. The most frequently used intravenous dose of alloxan in rats is 65mg/kg, but when it is administered intraperitoneally or subcutaneously its effective dose must be higher (Antia et al., 2005). This study concludes that, chemical induction of diabetes mellitus produced a stable and

sustainable level of hyperglycaemia in rats. The method has no differential effect on the response of antidiabetic agents and therefore is suitable for studying type 2 diabetes mellitus in experimental animals.

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