Instability of alloxan-induced diabetes and its impact on sex and thyroid hormones in male wistar rats-a pilot study

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Abstract

The relationship between diabetes mellitus and sex and/or thyroid hormones has been well documented in both human and animal studies ditto auto-reversibility of alloxan. However, the correlation between unstable diabetes and these hormones has little or no information in the literature; hence, the focus of this study. Diabetes was induced with a single intraperitoneal injection of 150 mg/kg of freshly prepared alloxan. Twenty-five adult male Wistar rats (weight 120-150 g) were used in this study. Alloxan was administered to 20 rats and 5 rats served as control. Alloxan-administered rats were further divided into two groups. One group (diabetic rats) was used as diabetic control and the other group served as reversed diabetic, which contained rats whose fasting blood glucose was confirmed to be normoglycemic post-diabetic. All rats were maintained on normal rat feed and water *ad libitum* and were monitored for 14 days. Blood glucose was monitored at intervals of 7 days after basal (before diabetes induction) and day 1(diabetes confirmation) values had been noted. Sex hormones: Luteinizing hormone (LH), follicle stimulating hormone (FSH), and estrogen (E) as well as thyroid hormones: Triiodothyronine (T3), tetraiodothyronine (T4) and thyroid stimulating hormone (TSH) were assayed after 14 days. Alloxan caused alteration in blood glucose levels of both diabetic and reversed diabetic groups. T4 level was lowered significantly in both diabetic (11.32 ± 0.26 μg/ml) and reversed diabetic (11.00 ± 0.16 μg/ml) groups in relation to the control. Other assayed hormones were not different significantly from the control. These findings indicate that influence of diabetes on these hormones may not be dependent on glucose gradation.

Keywords: Alloxan; Diabetes; Glucose; Hormones; Normoglycemic

1. Introduction

Alloxan and streptozotocin are the two most widely used chemicals to induce experimental diabetes. They both work by selectively destroying the beta cells of the pancreas. While streptozotocin destroys the beta cells by its alkylating potency, alloxan exerts its toxic effects on the beta cells via reactive oxygen species (ROS) generation and inhibition of glucokinase (Lenze, 2008). Nevertheless, the auto reversibility of the toxic effects of alloxan has been widely reported (Soni*et al.,* 2019). According to Soudamani *et al*. (2005); lowering of the epithelial diameter, luminal volume, stromal density of seminiferous tubules and plasma testosterone are

some ways by which alloxan causes reproductive dysfunctions in rats. Most of these changes are attributed to the ability of alloxan to inhibit the activities of some antioxidants such as superoxide dismutase and glutathione reductase in the testis in addition to its ability to increase testicular lipid peroxidation (El-Missiry, 1999).

Evidence abounds in the literature that thyroid hormones have profound effects on blood glucose levels. On the other hand, abnormal thyroid hormone levels have also been reported in diabetic patients with poor glucose control (Gursoy & Tuncel, 1999). Thyroid hormones have an opposite action to insulin in hepatic tissues. While insulin inhibits glucose production in the liver by inhibiting gluconeogenesis and glycogenolysis, thyroid hormones promote both pathways in the liver (Weinstein *et al.,* 1994). Reports have shown that thyroid hormones act synergistically with insulin by promoting glucose removal and utilization by peripheral tissues. They increase the expressions of Glut-4 and phosphoglycerate kinase genes that respectively participate in glucose transportation and glycolysis (Clement *et al.,* 2002; Viguerie, 2002).

Whereas several reports have shown the association between diabetes, sex, and thyroid hormones- there is little or no information in the literature about the impact of unstable alloxan-induced diabetes on sex and thyroid hormones. Hence, considering these relationships deserve to be given attention.

2. Methods

2.1 Animals

Twenty-five adults male Wistar rats weighing between 120-150 g were purchased from the National Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria. These rats were kept in laboratory cages (made of metal wire on all sides with wooden stand) and housed for two weeks to be acclimatized to the new environment. They were maintained under standard laboratory conditions (at 26±2°C temperature and 35±5% relative humidity) and exposed to equal amount of light/dark cycle (12 h/each). All animals had access to drinking water and food (produced by vital feeds, Bukuru, Jos, Nigeria) *ad libitum*. They were properly catered for following internationally accepted practices for use and care of laboratory animals, per US guidelines (NIH, 1992). At the end of the experiment, rats were fasted overnight and euthanized under moderate chloroform anesthesia.Registration and approval for this research was granted by the Departmental Ethics and Animal Welfare Committee of our institution in a letter with reference number: BCHEAWC-05/06/2019.

2.2 Chemical and Reagents

Alloxan was purchased from Zigma Aldrich, Jos Nigeria branch office. Normal saline was purchased from a Pharmacy shop, DutsinMa, Nigeria.

2.3 Induction of Diabetes

Fasted animals (overnight but with free access to water) were induced diabetes by intraperitoneal injection (by a single dose of 150 mg/kg body weight) of freshly prepared alloxan reconstituted with 0.9% normal saline (in a ratio of 10 mg/1 ml). Confirmation of diabetes (using a glucometer) was carried out on the 7^{th} day post alloxan injection. Animals with fasting blood glucose (mmol/L) more than double of the basal values were considered diabetic (Osibemhe *et al.*, 2017).

2.4 Experimental Design

In this study, twenty-five adult male Wistar rats (weight 120-150 g) were used. Alloxan was administered to 20 rats and 5 rats served as control. Rats administered with alloxan were further divided into two groups. One group (diabetic rats) consisted of 5 rats was used as diabetic control and another group (5 rats) served as reversed diabetic which contained rats that had confirmed fasting blood glucose as normoglycemic (within the range of basal glucose values) post-diabetic. This group was included in anticipation of reversal of the diabetic action of alloxan. Extra rats of the alloxan-injected that showed impaired glucose values (10 rats) were excluded from this study. All animals were monitored for 14 days. Blood glucose was monitored at intervals of 7 days after basal (before diabetes induction) and day 1 (day diabetes was confirmed) values had been noted. Sex hormones: Luteinizing hormone (LH), follicle stimulating hormone (FSH), and estrogen (E) and thyroid hormones: Triiodothyronine (T3), tetraiodothyronine (T4) and thyroid stimulating hormone (TSH) were assayed after 14 days. All analyses on each rat in each group were based on a single determination.

2.5 Collection and Preparation of Blood Sample for Analyses

At the end of the observation period, the rats were fasted overnight and euthanized under moderate chloroform anesthesia. Before death, blood was collected through the abdominal aorta using 5 ml syringe and kept in plain sample bottles. Samples were subsequently centrifuge at 3500 rpm for 15 min after proper clotting had been obtained and the serum was aspirated into plain sample containers (appropriately labeled with codes) for hormonal analysis.

2.6 Biochemical Analysis

Fasting blood glucose was monitored using a glucometer (accu-check). In this procedure, the tail of rats kept in a restrainer was cleaned with cotton wool that contains disinfectant (methylated spirit). And after massaging the tail, a syringe was used to prink the tip of the tail and a drop of blood was placed on the test strip already inserted in the glucometer (Osibemhe *et al.,* 2017). The result (mmol/L) displayed on the screen of the meter was recorded. Sex hormones: LH, FSH, and E and thyroid hormones: T3, T4, and TSH were assayed using fully automatic Finecare 3 Plus Immunoassay Analyzer (WondFo).

2.7 Sex Hormones and Thyroid Hormones Analysis

These hormones were assayed by using the fully automatic Finecare 3 Plus Immunoassay Analyzer (WondFo). The Finecare sex/thyroid hormones rapid qualitative test is based on fluorescence immunoassay (FIA) technique. The assay procedure involves five basic steps: preparation, sampling, mixing, loading and testing. In this analysis, the machine was prepared by activating it for "use" mode. This was followed by inserting into the machine an ID chip corresponding to the test Cartridge. Subsequently, 75 µL of each of the serum sample was transferred with the aid of a pipette into a Detection Buffer tube, the lid of the detection buffer was closed, and the sample was thoroughly mixed by shaking for about 10 mins. This was followed by pipetting 75 µL of each of the sample mixture and loading it into the sample well of the test Cartridge. By using the standard test mode, the test command was executed, and the results displayed on machine screen were printed.

2.8 Statistical Analysis

Data analyzed were presented as means ± SEM (n=5). One-way ANOVA followed by Duncan post hoc test to compare the means using the Statistical Package for the Social Sciences (SPSS) version 16. Statistically significant difference was set at $P<0.05$.

3. Results

3.1 Fasting Blood Glucose Level

Result of the fasting blood glucose levels is as shown in Figure 1. Data show no significant change in fasting blood glucose levels of the normal control rats from day 1-14 when compared with the basal values. The diabetic control had significant increase in fasting blood glucose level from day 1-14. However, the reversed diabetic rats exhibited increase in fasting blood glucose concentration on day 1 that normalized from day 7-14.

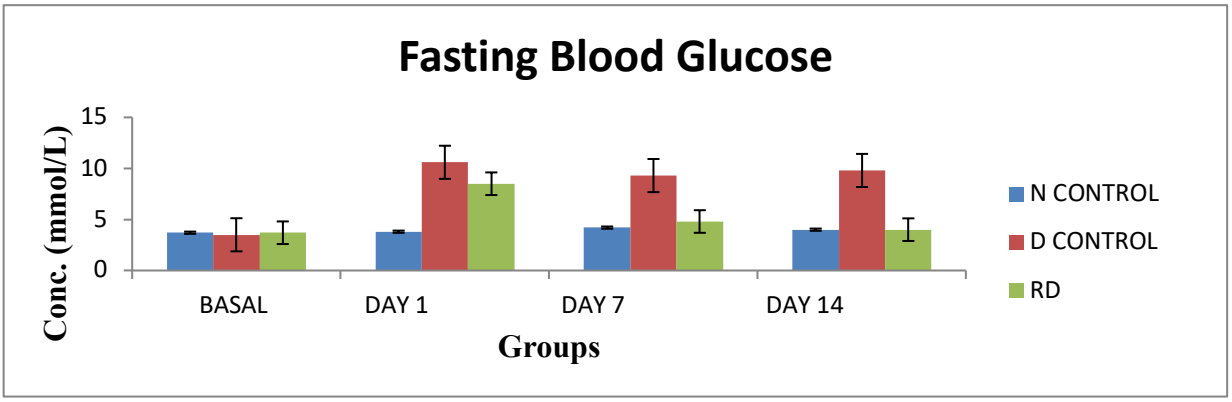

Fig. 1. Mean Fasting blood glucose concentration of diabetic and reversed diabetic rats fed with normal rat feed and monitored for 14 days. Values are expressed as concentration of glucose in mmol/L and are means ± SEM of n = 5. Significant difference was considered at $P \leq 0.05$ compared with basal (before diabetes induction). N CONTROL= normal control; D CONTROL = diabetic control; RD = reversed diabetic.

3.2 Sex Hormone

Data of the assayed sex hormones are as shown in Table 1. The results show no significant difference in the sex hormones (FSH, LH and E) analyzed in all the groups.

Table 1 Levels of sex hormones of male rats monitored for 14 days

Parameters Groups	FSH(MIu/ml)	LH(MIu/ml)	E(pg/ml)
Normal Control	95.00 ± 3.28	41.20 ±1.98	54.80±2.55
Diabetic Control	102.40 ±4.01	43.60 ±2.73	56.80 ±1.52
Reverse diabetic	100.40 ±6.24	42.40 ±4.61	60.40±1.69

Values are Means ± SEM (n=5).

3.3 Thyroid Hormone Levels

Table 2 shows the levels of the analyzed thyroid hormones: triiodothyronine (T3), tetraiodothyronine (T4) and thyroid stimulating hormone (TSH). T4 was significantly ($P \leq 0.05$) lowered in both the diabetic and reversed diabetic animals when compared with the normal control.

Table 2. Levels of thyroid hormone in male rats monitored for 14 days

PARAMETERS (serum)	**T3(ng/ml)**	**T4(µg/ml)**	**TSH(µU/ml)**
Normal Control	1.79 ± 0.03^{a}	12.02 ± 0.05^{b}	6.11 ± 0.36^{a}
Diabetic Control	1.66 ± 0.10^{a}	11.32 ± 0.26^{a}	6.50 ± 0.17^{a}
Reverse diabetic	1.81 ± 0.18^{a}	11.00 ± 0.16^{a}	6.79 ± 0.18^{a}

Values are Means ± SEM (n=5). Values with different superscript letters within each column represent significant difference ($P \leq 0.05$). While values with same letter "a" within each column represent no significant difference ($P \geq 0.05$).

4. Discussion

The relationships between diabetes, sex and thyroid hormones have been well documented. Diabetes has been reported to affect both hormones and the hormones can equally affect diabetes (Matsushita *et al.,* 2005; Hage *et al.,* 2011). Thyroid hormones are insulin antagonists in their response to glucose metabolism in the liver, and also work in synergy with insulin to effectively utilize glucose in peripheral tissues. The opposite and synergetic actions of insulin and thyroid hormones help to maintain glucose homeostasis under physiological conditions. Occurrence of hypothyroidism has been reported in type 1 diabetes mellitus; whereas hyperthyroidism tends to

constitute peripheral insulin resistance which progresses to diabetes (Pearce & Merriman, 2009; Kordonouri *et al.,* 2009). On the other hand, hyperthyroidism has been linked with normal, increased or reduced beta cell functions (Ortega *et al.,* 2008). Additionally, hypothyroidism has been reported to detonate a reduced rate of glucose production in the liver (Sol´a *et al.,* 2002).
On the correlation between sex hormones and diabetes, type 1 diabetes mellitus has been associated with testicular tissue and cell damage in experimental animals (Navarro-Casado *et al.,* 2010; Pavlinkova *et al.,* 2017). Whereas some existing literature have shown the association between diabetes, sex and thyroid hormones, there is a dearth of information about the impact of unstable alloxan- induced diabetes on sex and thyroid hormones levels.

In this study, the alterations in fasting blood glucose and the instability of alloxan observed, as presented in Figure 1 are not unusual as reports have demonstrated increased fasting blood glucose from alloxan injection (El-khamisy & Rezq, 2013; Amanda *et al.,* 2015); an action that is attributed to the capacity of alloxan to:1)-inhibit glucokinase enzyme and/or 2)-generate reactive oxygen species that in turn damages the β-cells of the pancreas leading to insulin deficiency and ultimately diabetes. These two mechanisms underlie the diabetogenicity of alloxan (Dunn & McLetchie, 1943; Jorns *et al.,* 1997; Osasenaga *et al.,* 2017). On the other hand, instability and auto-reversibility of alloxan as a diabetogenic drug has also been widely reported (Jain & Arya, 2011; Monika & Umme, 2012). The observed reversal of diabetes in this study may be attributed to the capacity of the pancreatic β-cells of the animals to regenerate (Dor *et al.,* 2004). Other possible reason for the observed reversal could be attributed to genetic polymorphism in drug metabolism within and between species (Pierre-Louis, 2010) or it may be as a result of differences in the expression of antioxidant enzymes. Glutathione has been reported to confer natural protection against the action of alloxan (Zhao *et al.,* 1987). Kubisch *et al.* (1997) and Tiedge *et al.* (1998) have also noted protection against alloxan action in transgenic mice with over expression of antioxidant enzymes. These possibilities may not be unconnected to the mechanism of action of alloxan. Diabetogenicity of alloxan has been attributed to the formation of reactive oxygen species (Dunn & McLetchie, 1943; Jorns *et al.,* 1997; Osasenaga *et al.,* 2017; Gisela, *et al.,* 2000). Other documented literature has also demonstrated the possibility of the toxicity and diabetogenicity of alloxan to vary widely among animals of the same species (Zhao *et al*., 1987; Monika & Umme, 2012).

Furthermore, the non-significant ($P \geq 0.05$) effects observed in the levels of the analyzed sex hormones in this study contradicts the findings of Ballester *et al.* (2004), who reported lowered levels of LH and FSH in diabetic rats. However, this result partly agrees with the findings of Hylmarova *et al.* (2020) who reported normal levels of FSH and estrogen in diabetic human males. Nevertheless, the normal levels of FSH and LH observed in this study may be attributed to uninterrupted level of estrogen balance. Hayes *et al.* (2001) have noted the regulation of gonadotropins secretion to be an association between the stimulation of gonadotropin releasing hormone (GnRH) from the hypothalamus and its inhibition by sex steroids (testosterone and estradiol) from the gonads. Other researchers have further narrowed the regulation of gonadotropins secretion predominantly to estrogen effect in both *in vitro* and *in*

vivo studies (Emons *et al.,* 1986; Fisher *et al.,* 1998; Hylmarova *et al.*, 2020).The distinctions in the findings of this research with other similar studies support the postulation by Ballester *et al.* (2004) who noted the regulation of testicular function in Wistar rats to be a result of multiple mechanisms involving the association between insulin/glucose with LH and FSH. We surmise that estrogen may also be a factor in these multiple regulatory mechanisms. However, the specific correlation between insulin/glucose and estrogen was not evaluated in this study and constitutes one of the study's setbacks.

The regulatory role of thyroid hormones on glucose homeostasis has earlier been noted. The results of the effect of diabetes/reversed diabetes on thyroid hormones in this study (Table 2) partly agree with the findings of Udiong *et al.* (2007), who noted normal levels of T3 and TSH in diabetic patients. The results also partly agree with the findings of Bharat *et al*. (2013), who reported lowered T4 levels in diabetic patients against non-diabetics. Abnormal thyroid hormone levels have also been reported in diabetic patients by Gursoy & Tuncel (1999). Abdul Azeem *et al.* (2021) have similarly reported decreases in thyroid hormone levels in alloxan induced diabetic male Wistar rats which were restored to normal upon stabilization of the blood glucose with γ-irradiated pumpkin seeds dried powder administration. The lowered T4 level observed in both reversed diabetic and diabetic animals' groups may reflect the selective effect of diabetes on thyroid hormone that may not be dependent on glucose gradation. This postulation is premised on the fact that, if hyperglycemia was the causation of decreases in the levels of thyroid hormones; it would be logical to have normal levels when the blood glucose stabilizes and/or abnormal levels when blood glucose is high. However, inconsistent findings have been reported in the levels of these hormones under diabetic conditions (Gursoy & Tuncel, 1999; Udiong *et al.*, 2007; Bharat *et al*., 2013) in both human and animal studies. Our postulation is further supported by the findings of Coiro *et al.* (1997) who recorded abnormality in the normal nocturnal TSH peak in type 1 diabetic patients even after amelioration in glycemic control.

5. Conclusions

This study, to our knowledge, has reported for the first time the influence of reversed diabetes on sex and thyroid hormones levels. Based on the findings, we surmise that diabetes exerts a selective effect on thyroid hormones that are not dependent on glucose gradation. In order to validate the postulations noted herein, we recommend that a prolonged study on the influence of diabetes/reversed diabetes on sex and thyroid hormones be carried out to make confirmed decisions. Also, the specific correlation between insulin/glucose and estrogen in the stimulation/inhibition of gonadotropins secretion needs to be investigated.

Abbreviations

GnRH: gonadotropin releasing hormone; ROS: reactive oxygen species; SEM: Standard error of mean.

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