Full Length Research Paper

The protective effects of Vitamin E and glibenclamide on spermatogenic and haematological changes in alloxan-induced diabetic male rats

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This study was designed to evaluate the preventive potentials of vitamin E and glibenclamide on spermatogenic and haematological damages in alloxan-induced diabetic male rats. Thirty mature male albino rats randomly assigned into 5 groups (n=6) were used for the study. Diabetes was experimentally induced in groups 1 to 4 while group 5 rats served as the negative control. Rats in groups 1, 2 and 3 were treated with glibenclamide, glibenclamide+Vitamin E, and Vitamin E, respectively while rats in group 4 served as the diabetic (positive) control. Results showed that percentage sperm motility was significantly higher (p<0.05) in groups 2 and 3 compared to group 1. Rats in group 2 recorded significant increases (p<0.05) in testicular weight, epididymal sperm reserve and testicular sperm reserve compared to groups 3 and 4, respectively. Rats of group 2 also had significantly higher packed cell volume and red blood cell counts compared to groups 1 and 3. Results from the study showed that combination of vitamin E and glibenclamide had optimum protective potential on the physiological parameters evaluated.

Key words: Diabetes, vitamin E, glibenclamide, spermatogenic changes, haematology, albino rats.

INTRODUCTION

Diabetes mellitus commonly referred to as diabetes is a disease associated with hyperglycemia due to insufficient production of insulin or inability of cells in the body to respond appropriately to available insulin (Rother, 2007). It affects man as well as animals. Dogs and cats are more affected among the animal species (Davidson, 2005; Rand and Marshall, 2005). Frequent urination, increased thirst, continuous quest for food and loss of weight are common symptoms of diabetes. Growing evidence has shown that diabetes impairs reproductive function in males. Diabetic male animals and man experience reduction in reproductive parameters, including reductions in weight of the testicles, number of motile sperm cells and sperm counts due to changes in
the testicles associated with sustained high blood glucose level (Orth et al., 1979; Paz and Homonnai, 1979; Hurtado de Catalfo et al., 1998; AbuAbeeleh et al., 2009). After two weeks of induction of diabetes with alloxan, diabetic male rats show decrease in reproductive function (Sanguinetti et al., 1995). Oxidative stress is the major cause of reproductive impairment in diabetic males (Zhao et al., 2011) because of excess reactive oxygen species in the body caused by high blood glucose level (Ballester et al., 2004).

Although glibenclamide is a standard antihyperglycemic agent used in the management of diabetes, there is paucity of information on its beneficial effects in addressing spermatogenic and haematologic changes in diabetics. Vitamin E ameliorates different types of reproductive toxicities in the body of animals (Brigelius and Traber, 1999; Kohen and Nyska, 2002). Hence, the aim of this study was to investigate the protective effects of vitamin E and glibenclamide on spermatogenic and haematologic changes in alloxan-induced diabetic male rats.

### MATERIALS AND METHODS

#### Animals

Thirty matured male Albino Wistar rats (Rattus norvegicus) of 12 weeks old weighing between 180 and 200 g were used for the study. The mature male rats (30) used for the study were obtained from the Department of Veterinary Anatomy, University of Nigeria, Nsukka. The rats were kept in cages at room temperature; feed and water were given to them ad libitum. The guidelines of Ward and Elsea (1997) and Zimmermann (1983) for use of animals for experiments were followed.

#### Ethical approval

Ethical approval was got from the ethical committee of Faculty of Veterinary Medicine, University of Nigeria, Nsukka (Approval Ref. No. 20170921).

#### Experimental design

The male albino rats were assigned into five groups (n=6). Diabetes was induced with alloxan (160 mg/kg) in groups 1 to 4 and treated daily for 3 weeks.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glibenclamide</th>
<th>Glibenclamide+ vitamin E</th>
<th>Vitamin E only</th>
<th>Diabetic control</th>
<th>Normal control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>46.33±0.67a</td>
<td>50.00±0.58b</td>
<td>44.67±0.33a</td>
<td>40.00±0.58c</td>
<td>54.00±0.58d</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.33±0.33ab</td>
<td>15.30±0.05ac</td>
<td>13.67±0.33bd</td>
<td>13.67±0.33b</td>
<td>15.44±0.33c</td>
</tr>
<tr>
<td>RBC (×10^6/ul)</td>
<td>6.80±0.43ac</td>
<td>8.60±0.36bc</td>
<td>6.23±0.25ad</td>
<td>5.90±0.06a</td>
<td>8.28±0.19c</td>
</tr>
<tr>
<td>WBC(×10^3/ul)</td>
<td>9.07±1.32a</td>
<td>10.25±1.78a</td>
<td>8.27±0.93a</td>
<td>2.15±0.55b</td>
<td>12.50±0.85a</td>
</tr>
</tbody>
</table>

*abcd* Showing significance among the various groups.

#### Induction of experimental diabetes

The rats were fasted for 12 h and fasting blood glucose evaluated before diabetes was induced (Venugopal et al., 1998). Alloxan (160 mg/kg) was injected into the rats intraperitoneally for diabetes to be induced. The levels of fasting blood glucose of the rats were evaluated daily. Blood glucose levels higher than 150 mg/dl in rats confirmed diabetes.

#### Evaluation of testicular weights

At the end of the study, the rats were sacrificed and the weight of the testicles was determined by weighing (Odo et al., 2018).

#### Gross percentage of sperm motility evaluation

Sperm sample was dropped on a pre-warmed clean slide from the epididymis; cover slip was placed on top of it and examined with a microscope at ×40 magnification. Motile sperms were counted and the percentage was determined (Hotchkiss et al., 1952).

#### Epididymal sperm reserve evaluation

The tails of the two epididymis were crushed in a ceramic mortar, mixed with normal saline (10 ml) and poured into a sieve. The filtrate (0.1 ml) was made up to 1 ml with 0.9 ml of white blood cells diluting fluid. This was then used to charge the Improved Neubauer chamber and examined with a microscope at ×40 magnification. Sperm cells were counted in 169 squares. This was then used to estimate the number of sperm cells in the original 10 ml of sperm solution (Amann, 1986).

#### Testicular sperm reserve evaluation

The two testicles were used according to the method of Amann (1986) already described.

#### Packed cell volume (PCV) evaluation

Haematocrit technique, according to Schalm et al. (1975), was used. Values were expressed in percentage (Table 1).
Figure 1. Mean percentage sperm motility.

Figure 2. Mean testicular weight.

Evaluation of total leukocyte and red blood cell counts

Haemocytometer technique according to Dacie and Lewis (1991) was used.

Determination of hemoglobin concentration

Hemoglobin concentration was evaluated according to the technique of Schalm et al. (1975) and values recorded in g/dl.

Data analysis

Data got from the study was statistically analyzed using one way analysis of variance (ANOVA). The variant means were separated using Duncan Multiple Range Test. Level of significant was accepted at p < 0.05.

RESULTS

Mean percentage sperm motility (Figure 1), testicular weight (Figure 2), testicular sperm reserve and epididymal sperm reserve (Figure 3) were significantly lower (p<0.05) in the diabetic control than in the treated groups and negative control in this study. There were significant increases in percentage sperm motility of group 2 (glibenclamide + vitamin E) and group 3 (vitamin E only) compared to group 1 (glibenclamide only). In rats treated with a combination of glibenclamide and vitamin E, the mean PCV and mean RBC were significantly increased compared to rats with either glibenclamide only or vitamin E only. There was a significant decrease in white blood cells of diabetic control compared to glibenclamide and glibenclamide + Vitamin E-treated groups.

DISCUSSION

The observed significant decreases (p<0.05) in mean percentage sperm motility, testicular weight, testicular sperm reserve and epididymal sperm reserve in the diabetic control compared to the treated groups and the negative control in this study agree with earlier reports.
Figure 3. Mean epididymal and testicular sperm reserves.

(Angel and Thliveris, 1986; Hurtado de Cattfo et al., 1998; Abuabeeleh et al., 2009). It is suggested that the significant decrease in testicular weight, testicular and epididymal sperm count of diabetic control may be due to sustained hyperglycemia and oxidative stress.

There were significant increases in percentage sperm motility of group 2 (glibenclamide + vitamin) (66.7±3.33) and group 3 (vitamin E only) (61.67±1.67) compared to group 1 (glibenclamide only) (43.33±3.33). This finding suggests that the decrease in sperm motility in glibenclamide-treated rats may be as a result of oxidative stress as glibenclamide lacks anti-oxidant property and therefore would not protect diabetic rats from oxidative stress. The sperm plasma membrane is very sensitive to oxidative stress attack and ongoing lipid peroxidation in diabetics since it contains a lot of unsaturated fatty acids. These unsaturated fatty acids create fluidity necessary for sperm motility (Olayemi, 2010). Vitamin E inhibits oxidative stress and lipid peroxidation in cellular microsomes and mitochondria (Lucosol et al., 1999; Gavazza and Catala, 2006). This may explain its ability to protect diabetic rats treated with glibenclamide + vitamin E and vitamin E in the study.

In rats treated with a combination of glibenclamide and vitamin E, the mean PCV and mean RBC were significantly increased when compared with rats with either glibenclamide only or vitamin E only. This gives an indication that both antihyperglycemic agent (glibenclamide) and antioxidant (vitamin E) should be administered for proper management of diabetes in males. Vitamin E has the ability to lower lipid peroxidation level which causes haemolysis of erythrocytes (Ashafa et al., 2009).

The significant decreases in PCV and Hb of the diabetic control as well as the vitamin E only treated group when compared with groups 1 and 2 may be due to the increased non-enzymatic glycosylation of RBC membrane proteins in diabetics due to sustained hyperglycemia (Oyedemi, 2011) since these groups did not receive glibenclamide. There was a significant decrease in white blood cells of diabetic control when compared with glibenclamide and glibenclamide + vitamin E-treated groups. This decrease may be a resultant effect of sustained hyperglycaemia observed in this group throughout the study period. Destruction of white blood cells occurs following sustained hypergaemia because diabetes suppresses the immune system of patients (Oyedemi, 2012).

Conclusion

Vitamin E has demonstrated significant protective potentials on spermatozoa and haematology in alloxan induced diabetic male rats treated with glibenclamide and hence established the physiological basis for its use as
one of the therapeutic agents in the management of diabetes mellitus in males.

RECOMMENDATION

In view of the findings from this study, supplementation of vitamin E is therefore recommended in diabetic males (animal and man) in addition to glibenclamide.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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