

ORIGINAL ARTICLE

Chronoefficacy of alpha-lipoic acid in combination with nifedipine and glimepiride attenuates testicular toxicity in diabetic rats

MICHAEL IKECHUKWU ORAEBOSI*¹, TEMIDAYO OLUTOYIN OLURISHE², SHERIFAT BOLA ANAFI² MOHAMMED BISALLA³

¹Department of Pharmacology and Therapeutics, Nile University of Nigeria Abuja

²Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria

³Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria

Abstract

Background: Knowledge of chronopharmacology and disease rhythms may provide additional therapeutic options for diabetic complications. The present research investigates the effect of chronomodulated alpha-lipoic acid/ nifedipine/ glimepiride combination in oxidative stress-mediated testicular toxicity in diabetic rats. A total of seven rat groups were used for the following study.

Methods: A group of non-diabetic rats and a group of diabetic rats were treated with 1 mL/kg of water to serve as normal and diabetic controls respectively. All other groups were diabetic and received 10 mg/kg glimepiride at 20:00h. Additionally, groups four to seven were treated with 20 mg/kg nifedipine at 08:00h while groups five to seven received additional treatments with alpha-lipoic acid (ALA) at 08:00h, 14:00h and 20:00h respectively. Rats were euthanized after four weeks of oral treatment and the epididymis and testis were excised for assessment of fertility markers. Serum testosterone and relative testes weights were measured. The right testes were preserved in phosphate buffer for cholesterol and antioxidant assay while the left testes were fixed in formalin for histological studies.

Results: All rat groups treated with ALA showed significantly ($p \leq 0.01$) better prognostic values for all markers assessed compared to the diabetic control group. Those treated with ALA at 20:00h showed better prognosis ($p \leq 0.05$) than treatment at other time points, showing values similar to the normal.

Conclusion: Time-dependent triple therapy with alpha-lipoic acid, nifedipine and glimepiride mitigates oxidative stress-mediated testicular injury in diabetic rats and its clinical benefits may be explored using equivalent circadian timing in men.

Keywords: antioxidants; circadian rhythm; diabetes; toxicology

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INTRODUCTION

Biological rhythms are ubiquitous in humans and regulate many biochemical and physiological endogenous processes [1]. These rhythms dictate manifestation of several diseases and drug actions by altering kinetics and dynamics of medications [2]. The rhythmic expression of diabetes mellitus is a consequence of circadian variation in glucose rhythm which peaks at the beginning of the activity period [3]. Diabetes mellitus is a global life-threatening disorder that requires adequate management. However, most conventional regimens do not put into consideration the rhythm of the disorder hence there is increased risk of severe complications which includes reproductive toxicities in men [4]. The effects on male fertility include impairment of spermatogenesis and low sperm count [4], reduced sperm motility and male sub-fertility [5], testicular and erectile dysfunction [6] and impairment of sperm DNA integrity [7].

Reactive oxygen species (ROS) mediated oxidative stress have been suggested to play a key role in the development of diabetic complications [8]. There is also evidence that ROS, endogenous antioxidants levels, and some of the diseases resulting from their imbalance such as diabetes, are expressed in rhythms [9]. Hence, management should employ therapy that will maintain a normal glucose level along with measures that will delay the progression or prevent complications and the use of antioxidants as complementary therapy. Furthermore, rhythms of the disease and that of the underlying mechanisms in addition to diurnal variations in drugs pharmacokinetics must be considered. Glimepiride has remained one of the drugs of choice used in the management of diabetes mellitus for over five decades [10, 11]. In addition to its cardiovascular benefits, nifedipine has shown promising antioxidant activity [12] and has also shown potential in ameliorating diabetic complications [13] Supplementation using alpha-lipoic acid (ALA) reduces glycated haemoglobin

*Correspondence to: Michael Ikechukwu Oraebosi, Ph.D. Candidate, Department of Pharmacology and Therapeutics, Nile University of Nigeria Abuja Plot 681, Cadastral Zone C-OO, Research & Institution Area Nigeria, Airport Road, Jabi 900001, Abuja, Nigeria. Tell: +2348033644434, Email: oraebosimichael@gmail.com

(HbA1c) in animal models [14] and also inhibits oxidative stress [15]. Advances in recent research shows the beneficial outcomes of chronotherapy in some diabetic complications [16, 17]. Hence, knowledge of circadian variation in oxidative stress and related testicular toxicity in diabetes may provide basis for newer therapeutic options. This may be achieved by modulation of times of administration of ALA as an adjunct in the management of type two diabetes mellitus (T2DM). This study investigates the effect of chronomodulated alpha-lipoic acid and nifedipine combination with concurrent glimepiride treatment in oxidative stress mediated testicular toxicity in diabetic rats.

Materials

Animals

Male rats of Wistar strain numbering sixty three and weighing between 150-155 g were used. The rats were purchased from McTemmy Laboratory Concept, Ogbomoso and kept in the Departmental animal house of Pharmacology and Therapeutics. They were kept in clean aluminium cages with wood shavings as bedding, with *ad libitum* water and rodent feed. Ethical directives (2010/63/EU) proposed by the European parliament on use and care of animals which complies with that of the Ahmadu Bello University, Zaria were observed.

Drugs

Alpha-lipoic acid (AO Pharmaceuticals, China), Nifedipine (Lek Pharmaceuticals, Slovenia), glimepiride (Sonafi pharmaceuticals Germany), chloroform, streptozotocin (Sigma chemical, Germany), 10 % dextrose in water (Juhel Nigeria Ltd, Enugu), formaldehyde solution (Sigma chemical, Germany).

METHODS

Induction of diabetes

Streptozotocin prepared in a citrate buffer solution of 0.1M concentration and a 4.5 pH was intraperitoneally administered at 50 mg/kg in 12 hours fasted rats. Water was replaced with 10 % dextrose in water for 24 hours to prevent hypoglycaemia resulting from pancreatic release of insulin [18]. Seven days post induction, assessment of fasting blood glucose was ensured with a glucometer and animals with blood glucose above 200 mg/dL were selected for grouping.

Experimental grouping

Seven rat groups participated in the research. A group of non-diabetic and another group of diabetic rats received treatment with 1 mL/kg of water to serve as normal control and diabetic control respectively. All other groups were diabetic receiving 10 mg/kg glimepiride at 20:00h (14 HALO). Additionally, groups four to seven were treated with 20 mg/kg nifedipine at 08:00h (2 HALO) while groups five to seven received additional treatments with ALA at 08:00h (2 HALO), 14:00h (8 HALO) and 20:00h (14 HALO) respectively. All drugs were administered orally for four weeks after which rats were euthanized and epididymis and testis were excised for assessment of some fertility markers. Relative organ weight of the testis were calculated and the right testis were preserved in phosphate buffer for testicular

cholesterol and antioxidant assay while all left testes were preserved in formalin for histological studies. Samples of blood were drawn after euthanasia and centrifuged to obtain serum from which testosterone levels were determined.

Semen analysis

Sperm suspension was obtained by carefully isolating and chopping the caudal end of the epididymis in a ten milliliters Petri dish containing two milliliters of normal saline maintained at 37 °C immediately after euthanasia. Sperm motility and count were assessed using the method of Belsey et al. [19] while viability was measured in 1% trypan blue reagent as described by Talbot and Chacon [20]. Sperm count, motility and viability were presented in millions/mL and percentages respectively. Sperm morphology was determined by using the method of Linder et al. [21]. A hundred sperm cells were investigated from each rat for deformities of tail and the head. The changes were determined by viewing under a light microscope and the percentage of head and tail defects were calculated. Sperm deformity index was calculated as follows:

$$\text{Sperm deformity} = \frac{\text{Total number of deformities}}{\text{Total number of sperm randomly selected and evaluated}}$$

Serum testosterone determination

Samples of blood were taken by means anticoagulant free vacutainers via the jugular veins and were spun in a centrifuge at 2000 g within 15 minutes. Levels of serum testosterone were determined using an enzyme linked free testosterone assay kit (DK0015 Diametra, Italy) according to the kit's protocol.

Antioxidant enzyme assays

The right testes were homogenized thoroughly in isolation medium. Levels of testicular antioxidant reserve such as superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) content and that of lipid peroxidation, malondialdehyde (MDA) were determined afterwards as described by Oraebosi et al. [22]; Obaji-Ogar et al. [36] and Mahadeshwara et al. [37].

Testicular cholesterol determination

This was carried out from the testicular homogenate by adopting the procedure of Zlatkis et al. [23]. This was done in test tubes by preparing 0.3 mL of the homogenate in 6 mL of 0.05% ferric chloride. 3 mL of sulphuric acid was afterwards added to the test tube containing mixture and left undisturbed for 20 minutes. An incubation mixture without the addition of homogenate was used as blank. Using a UV-VIS spectrophotometer (752N), absorbance was read at 540 nm and levels of testicular cholesterol were expressed in mg/g tissue weight.

Relative testicular weight

This was determined as shown in the expression below:

$$\text{Relative testes weight} = \frac{\text{weight of testis} \times 100}{\text{weight of rat of the day of euthanasia}}$$

Testicular histology

Preserved left testes were passed in graded concentration of alcohol and xylene before they were blocked in paraffin wax. Sections of tissue samples were then discoloured with haematoxylin and eosin and were read under a microscope to check the histoarchitecture [24].

Statistical analysis

Levene's test for homogeneity was done followed by Shapiro's checks for normality. Analyses were carried out with one way analysis of variance and Hochberg's *post hoc* with SPSS version 21. Values of *P* below or exactly 0.05 were measured as significant. The results are shown in tables as percentages or mean and standard error of mean, while histological results are in photomicrograph.

RESULTS

Time of day effects of ALA/nifedipine/glimepiride combination on sperm motility in diabetic rats

There was a significantly ($p \leq 0.01$) lower percentage of active cells in untreated diabetic rats in comparison to normal, with a greater percentage ($p \leq 0.01$) of sluggish and

non-motile cells respectively in comparison to the normal group. Concurrent treatment with glimepiride at 20:00h alongside nifedipine at 08:00h or with ALA at 08:00h, 14:00h and 20:00h produced significantly ($p \leq 0.01$) higher percentage active cells, lower sluggish and inactive cells in comparison to the untreated diabetic group. Drug treatment with 20:00h ALA however produced significantly ($p \leq 0.05$) better sperm motility than other time points. Table 1 shows the results.

Time of day effects of ALA/nifedipine/glimepiride combination on sperm viability and count in diabetic rats

The number of viable cells and sperm count were significantly ($p \leq 0.01$) higher in the group that received a combination of glimepiride at 20:00h and nifedipine at 08:00h or along with ALA at 08:00h 14:00h and 20:00h when compared to diabetic control. However the groups treated with a combination of glimepiride at 20:00h and nifedipine at 08:00h along with ALA at 20:00h produced better ($p \leq 0.05$) prognosis than with ALA treatment at 08:00h and 14:00h. The result is shown in Table 2.

Table 1. Time of day effects of ALA/nifedipine/glimepiride combination on sperm motility in diabetic rats

Groups	Active cells (%)	Sluggish cells (%)	Non-motile cells (%)
Non-diabetic control	96.55 ± 0.42	1.22 ± 0.27	1.88 ± 0.35
Diabetic control	0.00 ± 0.00**	6.00 ± 1.15**	94.00 ± 1.15**
Glimepiride 20:00h	4.00 ± 0.53**	13.57 ± 0.57**	83.00 ± 1.11**###
Glim2000+Nife0800	38.00 ± 1.01**###	33.60 ± 2.13**	30.40 ± 0.92**###
Glim2000+Nife0800+ALA0800	57.71 ± 3.7**###bc	22.28 ± 1.83**bc	20.00 ± 2.21**###bc
Glim2000+Nife0800+ALA1400	38.40 ± 1.66**###c	33.80 ± 1.83**c	27.80 ± 1.35**###c
Glim2000+Nife0800+ALA2000	77.28 ± 1.68###b	13.28 ± 0.86**b	10.00 ± 0.89**###b

Data are mean ± SEM, ** = $p \leq 0.01$ in comparison to normal, ^b = $p \leq 0.01$ in comparison to Glim0800+Nife0800, ^b = $p \leq 0.01$ compared to Glim0800+Nife0800, ^c = $p \leq 0.05$ compared to Glim0800+Nife0800+ALA2000 ### = $p \leq 0.01$ in comparison to untreated diabetic rats, one way analysis of variance with Hochberg's, n = 6-9, Glim2000+Nife0800 = received glimepiride and nifedipine at 20:00h and 08:00h respectively; ALA0800 = treated with alpha-lipoic acid at 08:00h; ALA1400 = treated with alpha-lipoic acid at 14:00h; ALA2000 = treated with alpha-lipoic acid at 20:00h

Table 2. Time of day effects of ALA/nifedipine/glimepiride combination on sperm viability and count in diabetic rats

Groups	Viable cells (%)	Non-viable cells (%)	Sperm count (millions)
Non-diabetic control	93.44 ± 0.85	6.55 ± 0.85	46.31 ± 0.43
Diabetic control	3.66 ± 0.49**	96.33 ± 0.49**	8.21 ± 0.21**
Glimepiride 20:00h	28.00 ± 0.81**###	72.00 ± 0.81**###	11.21 ± 0.24**#
Glim2000+Nife0800	59.40 ± 0.92**###	40.60 ± 0.92**###	26.60 ± 0.49**###
Glim2000+Nife0800+ALA0800	70.54 ± 0.64**###b	29.42 ± 0.64**###b	35.22 ± 0.80**###b
Glim2000+Nife0800+ALA1400	60.60 ± 1.07**###c	39.40 ± 1.07**###c	27.50 ± 0.80**###c
Glim2000+Nife0800+ALA2000	81.49 ± 0.89**###b	18.57 ± 0.89**###b	40.90 ± 0.56**###b

Data are mean ± SEM, ** = $p \leq 0.01$ in comparison to normal, ^b = $p \leq 0.01$ in comparison to Glim0800+Nife0800, ^b = $p \leq 0.01$ compared to Glim0800+Nife0800, ^c = $p \leq 0.05$ compared to Glim0800+Nife0800+ALA2000 ### = $p \leq 0.01$ in comparison to untreated diabetic rats, one way analysis of variance with Hochberg's, n = 6-9, Glim2000+Nife0800 = received glimepiride and nifedipine at 20:00h and 08:00h respectively; ALA0800 = treated with alpha-lipoic acid at 08:00h; ALA1400 = treated with alpha-lipoic acid at 14:00h; ALA2000 = treated with alpha-lipoic acid at 20:00h

Time of day effects of ALA/nifedipine/glimepiride combination on sperm morphology in diabetic rats

The results for head and tail defects are shown in Table 3. The percentage of head and tail defects in the untreated diabetic group was significantly ($p \leq 0.01$) higher in comparison to the normal rats. Various drug administration across all groups produced significantly ($p \leq 0.01$) lower percentages for both head and tail defects. However, the groups treated with a combination of glimepiride at 20:00h and nifedipine at 08:00h along with ALA at 08:00h and 20:00h produced significantly ($p \leq 0.01$) lower head and tail defects than treatment with a combination of glimepiride at 20:00h and nifedipine at 08:00h alone.

Time of day effects of ALA/nifedipine/glimepiride combination on testosterone, cholesterol and relative testes weight in diabetic rats

Mean levels of serum testosterone and testicular cholesterol with relative organ weight are shown in Table 4. These indices were significantly ($p \leq 0.01$) different in untreated diabetic animals in comparison to normal values. However, values for these indices were shown to ($p \leq 0.01$) produce significant amelioration in all groups receiving ALA in comparison to the diabetic control group. However, only

the group that received ALA at 20:00h in combination with glimepiride at 20:00h and nifedipine at 08:00h showed values similar to those of the non-diabetic control group.

Time of day effects of ALA/nifedipine/glimepiride combination on testicular oxidative stress and antioxidant markers

The results of analyses revealed that marker for lipid peroxidation (MDA) was significantly ($p \leq 0.01$) higher in the experimental group with significantly lower endogenous antioxidants reserve (SOD, CAT, GSH) in the untreated diabetic rats when compared to normal rats. All the groups treated with a combination of glimepiride at 20:00h and nifedipine at 08:00h along with ALA at 0800h and 2000h, respectively showed significantly ($p \leq 0.01$) lower MDA levels and higher ($p \leq 0.01$) endogenous antioxidant levels when compared to the untreated diabetic control group. In addition, rats which received ALA at 20:00h produced a better prognosis than other time periods. The results are shown in Table 5.

Time of day effects of ALA/nifedipine/glimepiride combination on testes histology in diabetic rats

Photomicrographic representation of diabetic rat with no drug treatment (Fig. 1B) showed testes without leydig cells

Table 3. Effect of chronomodulated ALA/nifedipine/glimepiride co-administration on sperm morphology in diabetic rats

Groups	Head defect (%)	Tail defect (%)	Sperm deformity index
Non-diabetic control	6.22 ± 0.43	5.22 ± 0.40	0.11±0.00
Diabetic control	31.33 ± 1.40**	31.00 ± 0.96**	0.62±0.03**
Glimepiride 20:00h	22.00 ± 0.81***	18.42 ± 0.89***	0.40±0.02***
Glim2000+Nife0800	17.80 ± 0.66***	14.60 ± 0.40***	0.32±0.01***
Glim2000+Nife0800+ALA0800	14.85 ± 0.26***b	12.57 ± 0.36***b	0.27±0.01***b
Glim2000+Nife0800+ALA1400	16.40 ± 0.67***	14.80 ± 1.15***	0.31±0.02***
Glim2000+Nife0800+ALA2000	14.14 ± 0.67***b	11.28 ± 0.68***b	0.25±0.01***b

Data are percentage of means and standard error of mean, ** = $p \leq 0.01$ in comparison to normal, ^b = $p \leq 0.01$ in comparison to Glim0800+Nife0800, ^b = $p \leq 0.01$ compared to Glim0800+Nife0800, ^{##} = $p \leq 0.01$ in comparison to untreated diabetic rats, one way analysis of variance with Hochberg's, n = 6-9, Glim2000+Nife0800 = received glimepiride and nifedipine at 20:00h and 08:00h respectively; ALA0800 = treated with alpha-lipoic acid at 08:00h; ALA1400 = treated with alpha-lipoic acid at 14:00h; ALA2000 = treated with alpha-lipoic acid at 20:00h

Table 4. Time of day effects of ALA/nifedipine/glimepiride combination on testosterone, cholesterol and relative testes weight in diabetic rats

Groups	Testosterone (ng/mL)	Cholesterol (mg/g)	Relative testes weight
Non-diabetic control	6.14 ± 1.57	6.95 ± 0.11	1.31 ± 0.93
Diabetic control	2.06 ± 0.07**	19.43 ± 0.18**	0.09 ± 0.91**
Glimepiride 20:00h	2.47 ± 0.12**	14.10 ± 0.71**	0.47 ± 0.08**
Glim2000+Nife0800	4.14 ± 0.10***	11.67 ± 0.41***	0.60 ± 0.49***
Glim2000+Nife0800+ALA0800	4.55 ± 0.13***	12.12 ± 0.34***b	1.12 ± 0.90***b
Glim2000+Nife0800+ALA1400	4.06 ± 0.19***	11.40 ± 1.07***	1.00 ± 0.20***
Glim2000+Nife0800+ALA2000	5.10 ± 0.15***b	6.17 ± 0.23***b	1.29 ± 0.11***b

Data are means and standard error of mean, ** = $p \leq 0.01$ in comparison to normal, ^b = $p \leq 0.01$ in comparison to Glim0800+Nife0800, ^b = $p \leq 0.01$ compared to Glim0800+Nife0800, ^{##} = $p \leq 0.01$ in comparison to untreated diabetic rats, one way analysis of variance with Hochberg's, n = 6-9, Glim2000+Nife0800 = received glimepiride and nifedipine at 20:00h and 08:00h respectively; ALA0800 = treated with alpha-lipoic acid at 08:00h; ALA1400 = treated with alpha-lipoic acid at 14:00h; ALA2000 = treated with alpha-lipoic acid at 20:00h

(L), sertoli cells (arrows), and presence central spermatozoa (asterisk). Other treatment groups (Fig., 1C-1F) showed some histological distortion, while treatment with

20:00h+nifedipine 08:00h+ALA20:00h showed historachitecture similar to that of normal rats. This is shown in Figure 1.

Table 5. Time of day effects of ALA/nifedipine/glimepiride combination on testicular oxidative stress and antioxidant markers

Groups	MDA (nmol/mg protein)	SOD (IU/mg protein)	CAT (U/g protein)	GSH (IU/mg protein)
Non-diabetic control	69.87 ± 1.84	18.33 ± 0.33	11.20 ± 0.27	17.11 ± 0.19
Diabetic control	199.66 ± 0.18**	6.15 ± 0.14**	1.48 ± 0.15**	4.79 ± 0.19**
Glimepiride 20:00h	191.71 ± 0.86**#b	7.17 ± 0.29**	4.57 ± 0.14**#b	5.22 ± 0.14**
Glim2000+Nife0800	92.88 ± 2.94**##	8.28 ± 0.36**##	7.06 ± 0.13**##	9.34 ± 0.18**##
Glim2000+Nife0800+ALA0800	84.90 ± 1.34**##c	12.01 ± 0.48**##b	8.10 ± 0.34**##b	12.08 ± 0.23**##b
Glim2000+Nife0800+ALA1400	89.48 ± 2.05**##	9.02 ± 0.15**##	10.92 ± 0.25**##	10.06 ± 0.31**##
Glim2000+Nife0800+ALA2000	75.65 ± 1.19##b	15.54 ± 0.18##b	7.74 ± 0.49##b	16.21 ± 0.17##b

Data are mean and standard error of mean, ** = $p \leq 0.01$ in comparison to normal, ^b = $p \leq 0.01$ in comparison to Glim0800+Nife0800, ^c = $p \leq 0.05$ in comparison to Glim0800+Nife0800, ## = $p \leq 0.01$ in comparison to untreated diabetic rats, one way analysis of variance and Hochberg's *post hoc* test, n = 6-9, Glim2000+Nife0800 = received glimepiride and nifedipine at 20:00h and 08:00h respectively; ALA0800 = treated with alpha-lipoic acid at 08:00h; ALA1400 = treated with alpha-lipoic acid at 14:00h; ALA2000 = treated with alpha-lipoic acid at 20:00h

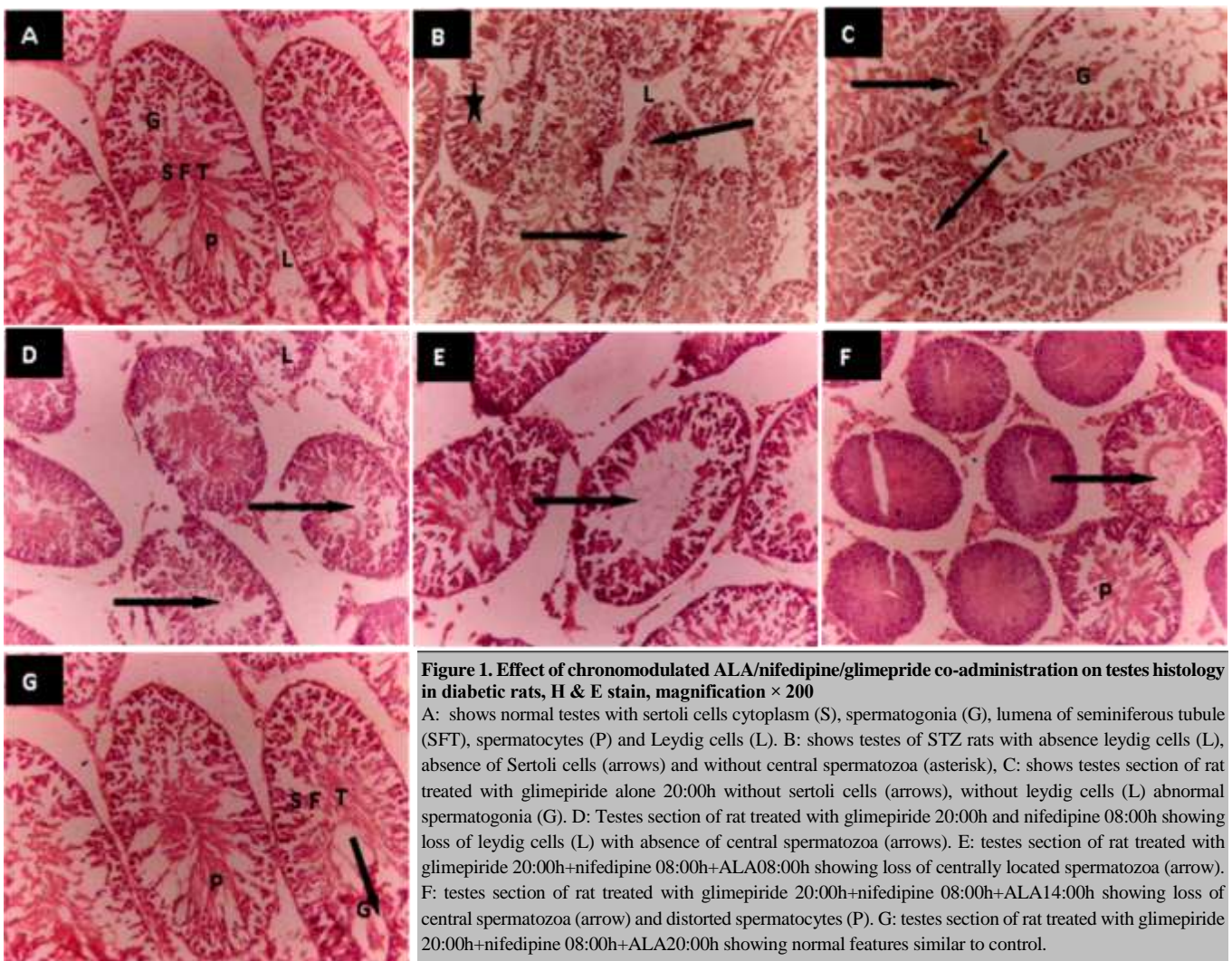


Figure 1. Effect of chronomodulated ALA/nifedipine/glimepiride co-administration on testes histology in diabetic rats, H & E stain, magnification × 200

A: shows normal testes with sertoli cells cytoplasm (S), spermatogonia (G), lumena of seminiferous tubule (SFT), spermatocytes (P) and Leydig cells (L). B: shows testes of STZ rats with absence leydig cells (L), absence of Sertoli cells (arrows) and without central spermatozoa (asterisk), C: shows testes section of rat treated with glimepiride alone 20:00h without sertoli cells (arrows), without leydig cells (L) abnormal spermatogonia (G). D: Testes section of rat treated with glimepiride 20:00h and nifedipine 08:00h showing loss of leydig cells (L) with absence of central spermatozoa (arrows). E: testes section of rat treated with glimepiride 20:00h+nifedipine 08:00h+ALA08:00h showing loss of centrally located spermatozoa (arrow). F: testes section of rat treated with glimepiride 20:00h+nifedipine 08:00h+ALA14:00h showing loss of central spermatozoa (arrow) and distorted spermatocytes (P). G: testes section of rat treated with glimepiride 20:00h+nifedipine 08:00h+ALA20:00h showing normal features similar to control.

DISCUSSION

A balance in testicular ROS and antioxidants is believed to be very essential for effective reproductive functions in men. Hence, oxidative stress in the testes resulting from an imbalance may be deleterious to reproductive health in men [25]. This balance may be maintained through secretion of endogenous antioxidants such as CAT, GPx, and SOD (MnSOD, CuSOD, ZnSOD and Cu/Zn-SOD). Although Zinc is a nonreactive redox metal, its role as an antioxidant is due to the activities of copper/zinc-superoxide dismutase (ZnSOD and Cu/Zn-SOD) which helps in membrane stabilization and increased metallothionein activities. Deficiencies in these antioxidant activities may result in unwanted health issues via ROS-antioxidants imbalance. Such imbalances have been reported in diabetes mellitus and may lead to severe complications. The involvement of oxidative stress in diabetes and its resulting complications is due to high plasma glucose levels overtime [9]. In diabetics, male fertility is affected by mechanisms that involve increased testicular ROS production [26], testicular testosterone deficiency caused by leydig cells destruction [27], and impaired spermatogenesis [28]. In this study, similar abnormalities were observed in untreated diabetic male rats may be a consequence of disturbance in spermatogenesis and sperm maturation impairments. These changes in addition to alterations in sperm viability, morphology, motility, testicular weights, and increased testicular cholesterol are important indices for male fertility [15]. They are often associated with increase in testicular oxidative stress. Hence therapies that reduce oxidative stress or improve endogenous antioxidants levels may be effective hindrances of the impairments of male fertility.

Although previous data have shown the benefits of ALA in mitigating testicular toxicity in rats by inhibiting oxidative stress [15], data from this study suggests that there is a time dependent variation in antioxidant activities of ALA in combination with nifedipine and glimepiride. This was identified after the mitigation of testicular toxicity in a group of rats was treated with ALA at night-time compared to other time points. This could be due to circadian variation in lipid peroxidation and endogenous antioxidant levels. The testes are known to possess antioxidant defence enzymes. However, their expression peaks during the dark phase in rats and it correlates with the acrophase of lipid peroxidation [29, 30]. In normal rats, these enzymes are able to cancel out the effects of emerging ROS, while oxidative stress results in diabetes due to imbalance. The values of lipid peroxidation and endogenous antioxidant as seen in the group treated with ALA at 20:00h appears to be similar to that of the non-diabetic control. This is an indication that ALA mitigated oxidative stress in this group of rats and it suggests that the drug's approximate peak plasma concentration after 20:00h administration matches with the levels of peak lipid peroxidation to prevent oxidative stress. This could be of clinical importance because ROS mediated oxidative stress damages sperm in patients with fertility problems. This may explain the observed improvement in semen analysis profile, testosterone and cholesterol levels, and testicular weights for

the group treated with ALA at 20:00h when compared to all other treatment groups.

The testis serves as a chief male reproductive organ whose function involves continuous production of spermatozoa in a process called spermatogenesis. Diurnal variation in spermatogenesis in rats has been reported to involve both cell division and cell differentiation [31]. It is reported that spermatogenesis increases at early rest periods with a peak production of spermatocytes at 06:00h [31] and reverses at the dark phase. In addition, exposure to long photoperiods in rats are reported to stimulate the development of reproductive and gonadal functions (increase testes weight, testosterone and luteinizing hormone levels) in contrast to a decrease that occurs during long dark period [32]. These reports imply that spermatogenesis peaks within the light phase with a trough at the dark phase. In this study, the restoration of reproductive markers followed the administration of ALA at 20:00h. This may suggest that ALA administration at this point (dark/rest phase) mitigated the processes responsible for a trough in reproductive markers in rats during the dark phase to keep approximate normal levels of reproductive parameters throughout the day. This is also supported by histological evidences where features from photomicrographs of the ALA 20:00h treated rats showed normal leydig cells, sertoli cells, spermatocytes, and spermatogonia. The role of these structures in reproduction has been reported. In rats, testosterone stimulates the release of prolactin [33]. Also, an increase in prolactin levels mediate testosterone release through an upregulatory effects of luteinizing hormone receptors on leydig cells [34]. ALA has been previously shown to enhance testosterone and prolactin levels in rats; hence the administration of ALA at 20:00h which falls within the time of their least expression [35] could have helped in regulating normal daily levels of these hormones and generating positive effects on reproductive parameters as seen in this study.

CONCLUSION

Time dependent triple therapy with alpha-lipoic acid, nifedipine, and glimepiride mitigates oxidative stress-mediated testicular damage in diabetic rats. The use of this combination in humans with approximate circadian timing may offer a new therapeutic approach for the treatment or prevention of testicular damage in diabetic males.

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REFERENCES

1. Akerstedt T, Gillberg T. The Circadian Variation of Experimentally Displaced Sleep. *Sleep* 1981;4:159-169.
2. Hermida RC, Ayala DE, Mojón A, Fernández JR. Influence of time of day of blood pressure-lowering treatment on cardiovascular risk in hypertensive patients with type 2 diabetes. *Diabetes Care* 2011;34:1270-1276.
3. Bolli GB, Gerich JE. The "Dawn-Phenomenon", a common occurrence in both non-insulin-dependent and insulin-dependent diabetes mellitus. *N Engl J Med* 1984;310:746-750.
4. Mangoli E, Talebi AR, Anvari M, Pouretezari M. Effects of experimentally-induced diabetes on sperm parameters and

- chromatin quality in mice. *Iran J Reprod Med* 2013;11: 53-60.
5. Singh S, Malini T, Rengarajan S, Balasubramanian K. Impact of experimental diabetes and insulin replacement on epididymal secretory products and sperm maturation in albino rats. *J Cell Biochem* 2009;108:1094-1101.
 6. Amaral S, Moreno AJ, Santos MS, Seica R, Ramalho-Santos J. Effects of hyperglycemia on sperm and testicular cells of Goto Kakizaki and streptozotocin treated rat models for diabetes. *Theriogenology* 2006;66: 2056-2067.
 7. Roessner C, Paasch U, Kratzsch J, Glander HJ, Grunewald S. Sperm apoptosis signalling in diabetic men. *Reprod Biomed Online* 2012;25:292-299.
 8. Baradaran A, Nasri H, Rafieian-Kopaei M. Comment on: antioxidative stress activity of *Stachys lavandulifolia* aqueous extract in humans. *Cell* 2013;15(3): 272-273.
 9. Kawahito S, Kitahata H, Oshita S. Problems associated with glucose toxicity: Role of hyperglycemia-induced oxidative stress. *World J Gastroenterol* 2009;15(33):4137-4142.
 10. Baynes HW. Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. *J Diabetes Metab.* 2015;6:541-548.
 11. Alhadramy MS. Diabetes and oral therapies: A review of oral therapies for diabetes mellitus. *J Taibah Uni Med Sc* 2016;11(4):317-329.
 12. Mak IT, Boehme P, Weqlicki WB. Antioxidant effects of calcium channel blockers against free radical injury in endothelial cells. Correlation of protection with preservation of glutathione levels. *Circ Res* 1992;70(6):1099-1103.
 13. Robertson S, Cameron ME, Cotter MA. Effect of calcium antagonist Nifedipine on peripheral nerve function in Streptozotocin induced diabetic rats. *Diabetologia* 1992;35(12) 1113-1117.
 14. Budin SB, Kee KP, Eng MY, Osman K, Bakar MA, Mohammed J. Alpha-lipoic acid prevents pancreatic islet cells Damage and dyslipidemia in streptozotocin-induced diabetic rats. *Malays J Med Sci* 2007;14(2):47-53.
 15. Prathima P, Venkaiah K, Pavani R, Daveedu T, Munikumar M, Gobinath M, Valli M, Sainath SB. α -lipoic acid inhibits oxidative stress in testis and attenuates testicular toxicity in rats exposed to carbimazole during embryonic period. *Toxicol Rep* 2017;4:373-381.
 16. Oraebosi MI, Olurische TO, Ayanwuyi LO. Chronomodulated Nifedipine Offers Reno-protection in Glimperide Treated Hyperglycaemic Rats. *J PharmaSciTech* 2016;6: (1) 40-44.
 17. Oraebosi MI, Olurische TO, Ayanwuyi LO. Chronomodulated Nifedipine Supports Concurrent Glimperide Administration with Subsequent Amelioration of Retinopathy and Peripheral Neuropathy in Diabetic Rats. *Egyptian J Basic Clin Pharmacol* 2019;9: doi:10.32527/2019/101437.
 18. Hussain HE, Jamil K, and Mala R. Hypoglycemic, hypolipidemic and antioxidant properties of Tulsi (*Ocimum sanctum* Linn) on streptozotocin induced diabetes rats. *Indian J Clin Biochem* 2001;16(2): 190-194.
 19. Belsey MA, Moghissi KS, Eliasson R, Paulsen CA, Gallegos AJ, Prasad MR. Laboratory Manual for the Examination of Human Semen and Semen-cervical Mucus Interaction, Press concern, Singapore 1980;54-55.
 20. Talbot P, Chacon. R.S. A triple-stain technique for evaluating normal acrosome reactions of human sperm. *J Exp Zool* 1981;215(2):201-208.
 21. Linder RE, Strader LF, Slott VL, Suarez JK. Endpoints of spermatotoxicity in the rat after short duration exposures to fourteen reproductive toxicants. *Reprod Toxicol* 1992;6:491-505.
 22. Oraebosi MI, Olurische TO, Anafi SB, Bisalla M. Chronopharmacology of the alpha-lipoic acid/nifedipine/glimperide combination in the amelioration of retinopathy in rats. *Chronobio int* 2021; 38(3): 443-450 <https://doi.org/10.1080/07420528.2020.1866004>.
 23. Zlatkis A, Zak B, Boyle AJ, Mich D (1953) A new method for the direct determination of serum cholesterol. *J Lab Clin Med* 2020;41:486-492.
 24. Arthur SJ, and John BA. colour Atlas of Histopathological Staining Techniques. Wolf MED.Pub. Ltd. London, 1978;pp 14-20.
 25. Sahoo DK, Roy A. Compromised rat testicular antioxidant defence system by hypothyroidism before puberty, *Int J Endocrinol* 2012;Article ID- 637825, <http://dx.doi.org/10.1155/2012/637825>.
 26. Ruder EH, Hartman TJ, Blumberg J, Goldman MB. Oxidative stress and antioxidants: exposure and impact on female fertility. *Hum Reprod Update* 2008;14(4):345-357.
 27. Khaki A, Khaki AA, Hajhosseini L, Golzer FS, Ainechi N. The antioxidant effects of ginger and cinnamon on spermatogenesis dysfunction of diabetes rats. *Afr J Trad Complement Altern Med* 2014;11(4):1-8.
 28. Arikawe AP, Daramola AO, Odofin AO, Obika LFO. Alloxan-induced and insulin-resistant diabetes mellitus affect semen parameters and impair spermatogenesis in male rats. *Afr J Reprod Health* 2006;10(3):106-113.
 29. Manoharan S, Kolanjiappan K. Diurnal rhythmicity of thiobarbituric acid reactive substances and antioxidants in experimental mammary carcinogenesis. *Exp Oncol* 2005;27(4): 298-302.
 30. Sani M, Sebai H, Ghanem-Boughanmi N, Boughattas NA, Ben-Attia M. Circadian (about 24-hour) variation in malondialdehyde content and catalase activity of mouse erythrocytes. *Redox Rep* 2015;20(1): 26-32.
 31. Möller U, Larsen JK, Hendl J, Christensen IJ. The cell kinetic effect of methotrexate on the epithelium of the hamster cheek pouch. A circadian dependent effect. In: 4th International Symposium on Flow Cytometry (O. D. Laerum, T. Lindmo, E. Thorud, eds.), Universitetsforlaget, Bergen, Oslo, Tromso, 1980;pp. 372-376.
 32. Gottreich A, Hammel I, Yogev L, Terkel J. Effect of photoperiod variation on testes and accessory sex organs in the male blind mole rat *spalax ehrenbergi*. *Life Sci* 2000;67(5):521-529.
 33. Kalra PS, Fawcett CP, Krulich L, Cann SMM. Effects of gonadal steroids on plasma gonadotropins and prolactin in the Rat. *Endocrinology* 197392(4):1256-1268.
 34. Poland V, Bartke A. Influence of endogenous prolactin on the luteinizing hormone stimulation of testicular steroidogenesis and the role of prolactin in adult male rats. *Steroids* 1988;51(5-6): 559-576.
 35. Beach JE, Trey L, Schomberg DW, Evertt JW. Nocturnal and diurnal levels of prolactin, LH, FSH, estrogens, and progesterones in middle aged, spontaneously persistent estrous rats. *Age* 1983;6:82-85.
 36. Obaji-Ogar LT, Nsonwa-Anyanwu AC, Odum FA. Oxidative DNA damage and pro-inflammatory response in chronic exposure to cement dust. *Asia Pac J Med Toxicol* 2020;9(1):3-10
 37. Mahadeshwara Prasad DRM, Kumar SH, Malini SSM, Shivanagappa M. Effects of diesel combustion nanoparticles on oxidative stress markers among the exposures. *Asia Pac J Med Toxicol* 2018;7:7-12.