

# The Effects of Wild African Potato (*Hypoxis hemerocallidea*) Supplementation on Streptozotocin-Induced Diabetic Wistar Rats Reproductive Function

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## Abstract

Sixty adult male Wistar rats (230-260 g) were randomly divided into 5 equal groups: normal, diabetic, diabetic +800 mg/kg *H. hemerocallidea*, diabetic+200 mg/kg *H. hemerocallidea* and non-diabetic+800 mg/kg *H. hemerocallidea*. Diabetes was induced with a single intraperitoneal injection of 50 mg/kg streptozotocin (STZ) and monitored during the study period. Blood glucose levels showed a significant increase ( $5.117 \pm 0.2412$  vs.  $30.70 \pm 1.2630$ ;  $p < 0.05$ ) 3 days after diabetes was induced using STZ. No mortality was observed during the study period. Blood samples, testes and epididymis were collected on the day of sacrifice. Testicular and epididymal lipid peroxidation (LPO) also showed a significant increase when diabetic control was compared to normal control. Body weights ( $315.7 \pm 9.348$  (g) vs.  $210.2 \pm 6.256$  (g);  $p < 0.05$ ), epididymis ( $1.005 \pm 0.0415$  (g) vs.  $0.7557 \pm 0.0279$  (g);  $p < 0.05$ ) and testicular ( $2.995 \pm 0.1179$  (g) versus  $2.140 \pm 0.0911$  (g);  $p < 0.05$ ) weights, sperm motility and morphology, superoxide dismutase, catalase, total glutathione, total antioxidant capacity, testosterone and estradiol levels ( $p < 0.05$ ) decreased in the diabetic Wistar rats. After 6 week administration of *H. hemerocallidea* there was a significant improvement in the above parameters in diabetic Wistar rats ( $p < 0.05$ ). In addition, the non-diabetic +800 mg/kg *H. hemerocallidea* group showed improvement in some sperm motility parameters, epididymal GSHT, testosterone and estradiol levels when compared to the normal control group ( $p < 0.05$ ). These results indicated that *H. hemerocallidea* supplementation is an effective approach to ameliorate male infertility in diabetic rats.

**Keywords:** Diabetes mellitus; *Hypoxis hemerocallidea*; Hypoglycaemic agents; Sperm

## Introduction

Infertility can be defined as the inability of a sexually active couple that is not using contraceptives, to achieve spontaneous pregnancy within at least one year of trying [1]. Statistics have shown that infertility affects 13-18% of couples worldwide. On reviewing the fertility status of modern society, it was revealed that decreased fertility rates are closely related to increased prevalence of DM [2,3]. Diabetes mellitus (DM) has been reported as one of the greatest global public health threats and its prevalence is rapidly increasing [4,5]. Hyperglycaemia, accompanying diabetes, has been linked to the overproduction of reactive oxygen species (ROS), also known as free radicals, consequently leading to oxidative stress (OS) that can impair the male reproductive function. ROS causes this by attenuating the body's natural defence mechanisms and inactivating antioxidant activity [6,7]. Antioxidants are molecules that acts as free radical scavengers acquired either endogenously in the body or exogenously by intake of certain foods. Antioxidants such as superoxide dismutase (SOD), catalase (CAT) and total glutathione (GSHT) are present in the seminal plasma and play an important role in assisting to neutralise ROS [8,9]. DM is an incurable disorder; therefore the aim of treatment is primarily to ensure longer life expectancy, relieving symptoms and preventing long-term complications [10,11]. This is normally achieved by lifestyle and dietary modification, insulin therapy and oral hypoglycaemic agents [11,12]. A reduced response as well as toxic side effects has been observed after prolonged treatment of DM with

pharmacological hypoglycaemic agents [12-14]. These observations have led to a search for alternatives of phytochemical origin.

*H. hemerocallidea* is commercially known as the African potato (AP) [15] that form part of the Hypoxidaceae family [16]. Infusions of the mature corms are frequently used in African traditional medicine for numerous ailments [17]. Aqueous extracts in mice and rats have demonstrated anti-inflammatory and anti-diabetic properties [18]. Several compounds have been extracted from *H. hemerocallidea* which may contribute to its therapeutic abilities. The primary compounds isolated were hypoxoside which gets converted by the gastrointestinal tract to rooperol, sterols, monoterpene glucosides and lectins. Apart from these, compounds such as polyphenols, steroids, glycosides, tannins, saponins and cytokinins have also been extracted from the corms of *H. hemerocallidea* [19,20].

From these facts it can be concluded that with the change in lifestyle this global increase in diabetes will have dire effects on reproductive health. The present study aims to observe whether *H. hemerocallidea* can inhibit the detrimental effects of oxidation and prevent damage to the reproductive system of diabetic male Wistar rats.

## Methods

### Experimental study

Sixty adult male Wistar rats were randomly allocated to 5 equal groups (12 rats per group): normal control, diabetic control, diabetic group treated with 800 mg/kg *H. hemerocallidea*, diabetic group treated with 200 mg/kg *H. hemerocallidea* and non-diabetic group

supplemented with 800 mg/kg *H. hemerocallidea*. The specific concentration of *H. hemerocallidea* were based on the average as well as double the concentrations used in the literature [17].

The experimental groups were:

A: Normal control group: Only fed with standard rat chow (SRC)

B: Diabetic control group: Diabetes induced using STZ and fed SRC

C: Diabetic+800 mg/kg *H. hemerocallidea*: Diabetic (STZ) supplemented with 800 mg/kg *H. hemerocallidea*

D: Diabetic+200 mg/kg *H. hemerocallidea*: Diabetic (STZ) supplemented with 200 mg/kg *H. hemerocallidea*

E: N+800 mg/kg *H. hemerocallidea*: Non-diabetic group supplemented with 800 mg/kg *H. hemerocallidea*

Institutional ethical clearance (NHREC: 230408-014) was obtained prior to commencing of the study. The weight of each rat was recorded at the beginning and the end of the study. After overnight fasting (12 hrs) diabetes was induced with a single 50 mg/kg intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (Sigma, USA) dissolved in 0.1 M cold citrate buffer pH 4.5 [21]. All rats were fed *ad lib* with standard rat chow and had free access to water daily. Blood glucose levels were measured using a portable Accucheck Glucometer (Roche, Germany) and the necessary amount of blood was collected via tail prick.

All animals received humane care according to the principles of Laboratory Animal Care of the National Society of Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals of the National Academy of Science.

### Sample collection, organ harvesting and tissue preparation

On completion of 6 weeks of treatment, rats were weighed and a fasting (12 hrs) blood glucose level was measured again with the tail prick method. The rats were anaesthetized with an intraperitoneal injection of 1ml ( $\pm$  60 mg/kg) sodium pentobarbitone. Blood was collected from the abdominal aorta into serum separator clot activator tubes (BD Vacutainers, Plymouth, UK), serum was obtained and stored at -80°C pending further analysis.

The epididymis and testes were excised and weighed before snap freezing one of each in liquid nitrogen and stored. The second epididymis was used to retrieve epididymal caudal sperm for motility analysis and preparing of sperm morphology slides.

### Sperm motility analysis

Sperm motility was performed by computer-aided sperm analysis (CASA) using the Sperm Class Analyser (SCA<sup>+</sup>; Microptic, Barcelona, Spain). The system analysed the total motility, progressive motility and kinematic and velocity parameters such as curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), amplitude of lateral head displacement (ALH), linearity (LIN), straightness (STR) and beat cross-frequency (BCF).

### Sperm morphology analysis

Sperm morphology was performed after air dried smears were stained using SpermBlue (SB) (Microptic SL, Barcelona, Spain). On completion of the fixing and staining procedures, the slides were

mounted using DPX mounting glue and cover-slipped. For morphometric evaluation, the Sperm Class Analyzer<sup>+</sup> (SCA<sup>+</sup>; Microptic, Barcelona, Spain) was used at 60X magnification using the blue filter. Rapid and accurate measurements of various variables were made and spermatozoa were accordingly classified as either normal or abnormal.

### Experimental Assays

1. Protein concentration of both epididymal and testicular tissue was measured by the Bradford method using bovine serum albumin as a standard [22].
2. Malondialdehyde (MDA) levels in epididymal and testicular tissue were determined spectrophotometrically using the same method as Devasagayam and co-workers [23].
3. SOD activity was determined using a modified method of Ellerby and Bredesen [24].
4. CAT activity was determined spectrophotometrically according to the method of Aebi [25].
5. Total GSH concentration was determined according to the method of Boyne and Ellman [26].
6. Ferric ion reducing antioxidant power (FRAP) is a colorimetric method used to measure the ferric reducing ability of biological samples by converting Fe<sup>3+</sup> into its oxidizing counterpart Fe<sup>2+</sup> as described by Benzie and Strain [27].
7. Oxygen radical absorbance capacity (ORAC) is used to measure the antioxidant scavenging activity of a substance. The procedure used is a modified method of Rautenbach and co-workers [28].
8. The testosterone rat/mouse Demeditec ELISA kit (DRG, Germany) was used for testosterone analysis according to the manufacturer's instructions.
9. The Estradiol Demeditec ELISA kit (DRG, Germany) was used for analysis according to the manufacturer's instructions.

### Statistical analysis

The results were statistically analysed using PRISM 5 software and presented as mean with standard error of mean (SEM). The significance of differences among groups was analysed statistically by one-way ANOVA (analysis of variance), followed by Student's unpaired t-test. The differences were considered statistically significant if  $p < 0.05$  and results presented as mean  $\pm$  SEM.

### Results

#### Blood glucose results of Wistar rats

After 12 hrs of food deprivation fasting blood glucose levels of all 5 groups were measured. Initial fasting glucose levels, glucose levels 3 days after STZ injection and glucose levels after 6 weeks of treatment with *H. hemerocallidea* was taken. The diabetic control showed a significant increase ( $p < 0.05$ ) in glucose levels 3 days after DM was induced and on completion of the 6 weeks when compared to initial fasting glucose levels.

There was also a significant increase ( $p < 0.05$ ) when glucose levels after the 6 week period was compared to glucose levels initially taken from the diabetic group treated with 800 mg/kg and 200 mg/kg *H. hemerocallidea* (Table 1).

Groups/Treatment	Fasting glucose results (mM/l)	Blood glucose after 3 days without treatment (mM/l)	Blood glucose after 6 weeks after treatment (mM/l)
A: Normal control group	4.583 ± 0.1036	5.117 ± 0.2412*#	4.73 ± 0.1034*#
B: Diabetic control group	4.120 ± 0.1812	30.70 ± 1.2630 <sup>§</sup>	26.32 ± 1.4220 <sup>§</sup>
C: Diabetic+800 mg/kg <i>H. hemerocallidea</i>	4.280 ± 0.1855	29.76 ± 1.6750 <sup>§</sup>	9.38 ± 1.9410* <sup>§</sup>
D: Diabetic+200 mg/kg <i>H. hemerocallidea</i>	3.767 ± 0.1509	31.75 ± 0.8920 <sup>§</sup>	15.33 ± 2.673* <sup>§</sup>
E: Non-diabetic+800 mg/kg <i>H. hemerocallidea</i>	4.175 ± 0.1620	4.692 ± 0.1252*#	4.49 ± 0.1525*#

Data presented as mean ± SEM, (\*) p<0.05 compare to Diabetic control, (#) p<0.05 compare to Diabetic+200 mg/kg *H. hemerocallidea*, (°) p<0.05 compare to Non-diabetic supplemented with 800 mg/kg *H. hemerocallidea*, n=12 per group.

**Table 1:** Blood glucose levels of Wistar rats before induction of diabetes, 3 days after diabetes was induced and 6 weeks after treatment.

### Body weights

A significant decrease (p<0.05) in body weights was observed when the diabetic control group was compared to initial weights taken, normal control group and non-diabetic group+800 mg/kg *H. hemerocallidea*.

The diabetic group treated with 800 mg/kg *H. hemerocallidea* showed a significant increase (p<0.05) when compared to the diabetic control group and diabetic group+200 mg/kg *H. hemerocallidea* (Table 2).

### Epididymal and testicular weights

A significant difference (p<0.05) was observed in epididymal and testicular weights when the normal control group, non-diabetic group supplemented with *H. hemerocallidea* and the diabetic groups treated with *H. hemerocallidea* was compared to the diabetic control group (Table 2).

### Epididymal sperm motility and morphology

Both sperm motility and morphology showed the same trends when groups were compared to each other. The diabetic control group displayed a significant decrease (p<0.05) when compared to the

normal control group, diabetic 800 mg/kg and non-diabetic+800 mg/kg *H. hemerocallidea*.

However, no significant difference (p>0.05) in sperm motility and morphology was observed between the diabetic control group and the diabetic group treated with 200 mg/kg *H. hemerocallidea*.

A significant increase (p<0.05) was observed in sperm motility and morphology of diabetic groups treated with 800 mg/kg *H. hemerocallidea* was compared to the diabetic control group (Table 3).

### Lipid peroxidation in epididymal and testicular tissue

Epididymal and testicular MDA levels were significantly increased (p<0.05) when the diabetic control was compared to the normal control group. There was also a significant increase (p<0.05) when the diabetic control group was compared to the diabetic groups treated with *H. hemerocallidea* and the non-diabetic group supplemented with 800 mg/kg *H. hemerocallidea*. Epididymal MDA were significantly decreased (p<0.05) when the normal control group was compared to non-diabetic group supplemented with 800 mg/kg *H. hemerocallidea*. A significant decrease (p<0.05) was also observed when the diabetic group treated with 800 mg/kg *H. hemerocallidea* was compared to 200 mg/kg *H. hemerocallidea* (Table 4).

Groups/Treatment	Initial Body weights (g)	Body weights after 6 weeks (g)	Epididymal weights (g)	Testicular weights (g)
A: Normal control	215.0 ± 3.236	315.7 ± 9.348*#	1.005 ± 0.0415*	2.995 ± 0.1179*
B: Diabetic control	213.3 ± 3.073	210.2 ± 6.256	0.7557 ± 0.0279* <sup>§</sup>	2.140 ± 0.0911* <sup>§</sup>
C: Diabetic+800 mg/kg <i>H. hemerocallidea</i>	213.3 ± 3.945	239.3 ± 14.47*#	0.9777 ± 0.0404*	2.868 ± 0.0718*
D: Diabetic+200 mg/kg <i>H. hemerocallidea</i>	213.9 ± 2.799	218.4 ± 4.936 <sup>§</sup>	0.9173 ± 0.0357*	2.744 ± 0.1077*
E: Non-diabetic+800 mg/kg <i>H. hemerocallidea</i>	226.3 ± 2.801	303.8 ± 7.567*#	1.030 ± 0.0297*	3.116 ± 0.1443*

Data presented as mean ± SEM;  
 (\*) p<0.05 compare to Diabetic control;  
 (#) p<0.05 compare to Diabetic+200 mg/kg *H. hemerocallidea*;  
 (°) p<0.05 compare to Non-diabetic supplemented with 800 mg/kg *H. hemerocallidea*;  
 n=12 per group.

**Table 2:** Body weights, epididymal and testicular weights of Wistar rats.

### Antioxidant enzyme activities of epididymal and testicular tissue

The assessment of antioxidants showed lower concentrations in epididymal and testicular SOD, CAT and GSht levels when the diabetic control group was compared to the normal control group. The diabetic control group showed a significant decrease ( $p < 0.05$ ) in testicular and epididymal SOD, CAT and GSht levels when compared to diabetic groups treated with *H. hemerocallidea*.

However, there was no significant difference when testicular SOD of the diabetic control group was compared to the diabetic group treated with 800 mg/kg *H. hemerocallidea*. A significant decrease ( $p < 0.05$ ) in testicular SOD and GSht was observed and a significant decrease ( $p < 0.05$ ) of testicular CAT was observed when normal control group was compared to the non-diabetic group supplemented with 800 mg/kg *H. hemerocallidea* (Table 4).

Sperm motility parameters	Group/ Treatment				
	A: Normal control	B: Diabetic control	C: Diabetic+800 mg/kg <i>H. hemerocallidea</i>	D: Diabetic+200 mg/kg <i>H. hemerocallidea</i>	E: Non-diabetic+800 mg/kg <i>H. hemerocallidea</i>
Fast progressive (%)	46.5 ± 6.670 <sup>*#§</sup>	14.0 ± 5.077 <sup>§</sup>	46.0 ± 8.407 <sup>*#§</sup>	14.1 ± 3.521 <sup>#</sup>	50.8 ± 6.117 <sup>*#</sup>
Slow progressive (%)	30.7 ± 6.711 <sup>*§</sup>	46.5 ± 11.888 <sup>#§</sup>	30.4 ± 14.474 <sup>*#</sup>	50.1 ± 15.814 <sup>*§</sup>	29.1 ± 4.881 <sup>*#</sup>
Non-progressive (%)	22.8 ± 2.811 <sup>*#§</sup>	39.5 ± 7.619 <sup>§</sup>	23.5 ± 2.826 <sup>*#§</sup>	35.8 ± 6.477 <sup>§</sup>	20.0 ± 1.266 <sup>*#</sup>
Total progressive (%)	80.3 ± 9.608 <sup>*#§</sup>	33.3 ± 5.376 <sup>#§</sup>	69.5 ± 13.899 <sup>*#§</sup>	35.7 ± 5.899 <sup>*§</sup>	79.8 ± 10.837 <sup>*#</sup>
Immotile (%)	19.7 ± 2.677 <sup>*#</sup>	66.7 ± 7.138 <sup>§</sup>	30.5 ± 7.290 <sup>*#§</sup>	64.3 ± 8.930 <sup>§</sup>	20.2 ± 1.927 <sup>*§</sup>
VCL (m/s)	193.1 ± 8.526 <sup>*#</sup>	115.5 ± 8.850 <sup>#§</sup>	188.6 ± 7.218 <sup>*#</sup>	133.9 ± 9.398 <sup>*§</sup>	187.5 ± 12.20 <sup>*#</sup>
VSL (m/s)	49.65 ± 1.943	36.76 ± 2.140 <sup>§</sup>	48.84 ± 1.613	43.30 ± 2.388	51.64 ± 1.525 <sup>*</sup>
VAP (m/s)	76.88 ± 5.399 <sup>*</sup>	58.83 ± 3.118 <sup>#§</sup>	83.42 ± 3.826 <sup>*</sup>	71.12 ± 3.902 <sup>*</sup>	83.78 ± 2.583 <sup>*</sup>
LIN (%)	27.79 ± 1.249	29.56 ± 1.353	27.04 ± 1.110	30.30 ± 2.385	27.82 ± 1.179
STR (%)	59.56 ± 1.659	61.18 ± 1.378	58.76 ± 1.852	63.88 ± 2.005	53.88 ± 5.521
Wobble (%)	45.85 ± 0.485	48.73 ± 1.339	45.98 ± 1.210	46.04 ± 3.032	45.98 ± 0.595
ALH (m)	11.99 ± 0.7532	9.488 ± 0.4037	11.38 ± 0.5704	8.675 ± 1.286	11.66 ± 0.7160
BCF (Hz)	3.350 ± 0.1875	3.543 ± 0.2599	3.640 ± 0.2977	3.125 ± 0.5031	3.400 ± 0.2324
<b>Sperm Morphology</b>					
Normal (%)	92.67 ± 2.917 <sup>*#</sup>	69.33 ± 4.723 <sup>§</sup>	88.67 ± 4.185 <sup>*#</sup>	70.60 ± 8.727 <sup>§</sup>	81.60 ± 3.187 <sup>*#</sup>
Abnormal (%)	7.333 ± 2.917 <sup>*#§</sup>	35.60 ± 4.534	16.50 ± 3.403 <sup>*</sup>	24.50 ± 9.323	18.40 ± 3.187 <sup>*</sup>

Data presented as mean ± SEM, (\*)  $p < 0.05$  compare to Diabetic control, (#)  $p < 0.05$  compare to Diabetic+200 mg/kg *H. hemerocallidea*, (§)  $p < 0.05$  compare to Non-diabetic +800 mg/kg *H. hemerocallidea*, n=12 per group.

**Table 3:** Progression and velocity parameters as well as morphology of spermatozoa of normal control (A), diabetic control (B), diabetic group treated with 800 mg/kg *H. hemerocallidea* (C), diabetic group treated with 200 mg/kg *H. hemerocallidea* (D) and non-diabetic group supplemented with *H. hemerocallidea* (E) after 6 week treatment.

### Total antioxidant capacity (TAC) of epididymal and testicular tissue

On assessing epididymal FRAP and ORAC was significantly decreased ( $p < 0.05$ ) in the diabetic control group when compared to the normal control group. A significant decrease ( $p < 0.05$ ) was also observed when the diabetic control group was compared to the diabetic group treated with 800 mg/kg and the diabetic group treated with 200 mg/kg *H. hemerocallidea* as well as when compared to the non-diabetic group supplemented 800 mg/kg *H. hemerocallidea*.

There was also a significant decrease in epididymal ORAC ( $p < 0.05$ ) when the diabetic groups treated with 800 mg/kg and 200 mg/kg *H. hemerocallidea* was compared to the non-diabetic group supplemented with 800 mg/kg *H. hemerocallidea*. A significant decrease ( $p < 0.05$ ) was

observed when testicular FRAP and ORAC of the normal control group was compared to the diabetic control group.

It was also observed that there was a significant difference ( $p < 0.05$ ) in testicular FRAP and ORAC when the diabetic group treated with 800 mg/kg *H. hemerocallidea* was compared to the diabetic group treated with 200 mg/kg *H. hemerocallidea* (Table 4).

### Serum testosterone an estradiol analysis

Serum levels of testosterone and estradiol were significantly decreased ( $p < 0.05$ ) in the diabetic control group when compared to the normal control group. However, a significant increase ( $p < 0.05$ ) was observed when the diabetic group treated with 800 mg/kg *H. hemerocallidea* and the non-diabetic group supplemented with 800

mg/kg *H. hemerocallidea* was compared to the diabetic control group. A significant increase ( $p < 0.05$ ) in testosterone and estradiol was also observed when the non-diabetic group supplemented with 800 mg/kg *H. hemerocallidea* was compared to the normal control group and diabetic group treated with 800 mg/kg *H. hemerocallidea* (Table 4).

## Discussion

Evidence suggesting that diabetes has adverse side effects on the male reproductive function is on the increase [29]. OS has shown to play a major role in these observations [30]. This study investigated the

effects of *H. hemerocallidea* on the reproductive function of STZ-induced diabetic Wistar rats.

Hyperglycaemia is a known characteristic of DM resulting in an increase of ROS and reactive nitrogen species (RNS) (collectively known as free radicals) thereby leading to OS. Humans have defensive mechanisms in the form of endogenous and exogenous antioxidants to deal with excessive free radical formation that can cause oxidative damage. However, when the rate of formation of ROS overwhelms the detoxifying ability of the antioxidants, OS can occur [31-33].

Sample types and Tests	Group/ Treatments				
	Normal control group	Diabetic control group	Diabetic+800 mg/kg <i>H. hemerocallidea</i>	Diabetic+200 mg/kg <i>H. hemerocallidea</i>	Non-diabetic+800 mg/kg <i>H. hemerocallidea</i>
<b>Epididymal</b>					
MDA (µmol/g protein)	0.2116 ± 0.0045 <sup>*</sup>	0.265 ± 0.0112 <sup>#§</sup>	0.1782 ± 0.0062 <sup>*</sup>	0.2122 ± 0.0115 <sup>*§</sup>	0.1609 ± 0.0076 <sup>#</sup>
SOD (µmol/mg protein)	24.60 ± 0.4689 <sup>#</sup>	21.33 ± 0.8898 <sup>#§</sup>	27.40 ± 0.3502 <sup>*§</sup>	27.80 ± 0.3980 <sup>*§</sup>	24.73 ± 0.6194 <sup>#</sup>
CAT (µmol/L)	0.2183 ± 0.0060 <sup>#</sup>	0.1950 ± 0.0065	0.2143 ± 0.0065 <sup>#</sup>	0.1900 ± 0.0091 <sup>§</sup>	0.2075 ± 0.0085 <sup>#</sup>
GSHt (µmol/ml)	126.4 ± 1.330 <sup>*§</sup>	119.1 ± 1.122 <sup>#§</sup>	130.3 ± 2.092 <sup>*</sup>	131.1 ± 1.464 <sup>*</sup>	132.0 ± 1.095 <sup>*</sup>
FRAP (µmol/L)	746.5 ± 52.67 <sup>*</sup>	364.6 ± 93.85 <sup>*§</sup>	694.6 ± 63.34 <sup>*</sup>	661.1 ± 35.41 <sup>*</sup>	681.1 ± 57.05 <sup>*</sup>
ORAC (µmolTE/ml)	303.3 ± 6.321 <sup>*#§</sup>	169.8 ± 14.45	142.4 ± 5.251 <sup>§</sup>	148.4 ± 3.533 <sup>§</sup>	183.6 ± 4.987 <sup>#</sup>
<b>Testicular</b>					
MDA (µmol/g protein)	0.0425 ± 0.0023 <sup>*</sup>	0.0562 ± 0.0013 <sup>#§</sup>	0.0428 ± 0.0015 <sup>*</sup>	0.0447 ± 0.0031 <sup>*</sup>	0.0471 ± 0.0014 <sup>*</sup>
SOD (µmol/mg protein)	38.47 ± 0.8523 <sup>*#§</sup>	20.35 ± 0.4694	22.67 ± 0.5435	23.93 ± 0.4491 <sup>*</sup>	25.07 ± 1.530 <sup>*</sup>
CAT (µmol/L)	0.5760 ± 0.0397 <sup>#§</sup>	0.2100 ± 0.0228 <sup>#</sup>	0.3929 ± 0.0281 <sup>*</sup>	0.3577 ± 0.0165 <sup>*</sup>	0.2967 ± 0.0088
GSHt (µmol/ml)	123.9 ± 2.150 <sup>*</sup>	103.3 ± 2.407 <sup>#§</sup>	133.9 ± 1.060 <sup>#</sup>	141.6 ± 0.9625 <sup>*§</sup>	132.4 ± 0.9051 <sup>#</sup>
FRAP (µmol/L)	682.7 ± 40.55 <sup>*§</sup>	603.7 ± 41.15 <sup>§</sup>	480.8 ± 52.02 <sup>*#§</sup>	627.0 ± 47.15 <sup>§</sup>	501.9 ± 37.65 <sup>*#</sup>
ORAC (µmolTE/ml)	169.7 ± 4.543 <sup>*</sup>	145.0 ± 2.098 <sup>#§</sup>	180.7 ± 2.850 <sup>*</sup>	175.2 ± 2.647 <sup>*</sup>	164.6 ± 3.767 <sup>*</sup>
<b>Serum</b>					
Testosterone (ng/ml)	1.023 ± 0.0590 <sup>*§</sup>	0.7466 ± 0.0210 <sup>§</sup>	0.9795 ± 0.0209 <sup>*§</sup>	0.8778 ± 0.0284 <sup>§</sup>	1.381 ± 0.0588 <sup>#</sup>
Estradiol (pg/ml)	1.641 ± 0.0242 <sup>§</sup>	1.530 ± 0.0354 <sup>§</sup>	1.765 ± 0.0284 <sup>*#</sup>	1.601 ± 0.0213 <sup>§</sup>	1.856 ± 0.0226 <sup>#</sup>

Data in table is presented as mean ± SEM. (\*) represents a significant difference when compared to Diabetic control, (#) represents a significant difference when compared to Diabetic group+200 mg/kg *H. hemerocallidea* and (§) represents significance when compared to Non-diabetic+800 mg/kg *H. hemerocallidea*, n=12 per group.

**Table 4:** Epididymal, testicular and serum results of Wistar rats after a 6 week period of treatment.

Excessive ROS and subsequent OS can thus cause structural deterioration and instability of macromolecules such as carbohydrates, proteins, lipids and DNA, consequently affecting proper cellular signalling pathways, gene regulation and cellular function [6,31].

After the 6 week treatment period the diabetic control group showed an increase in blood glucose levels as well as in LPO activity when compared to normal controls. Decreased levels of body, epididymal and testicular weight, decreased sperm motility and sperm morphology, decreased levels of antioxidants (SOD, CAT and GSHt),

decrease TAC (FRAP and ORAC) as well as a decrease in testosterone and estradiol concentrations (Table 4) [34-36] were also observed when compared to the normal control. From these results it was evident that the antioxidant capacity of these diabetic animals was significantly reduced. This more than likely led to a ROS: TAC imbalance and the onset of OS. The pathological levels of ROS, amongst others, attack the polyunsaturated fatty acids (PUFAs) in the sperm plasma membranes as spermatozoa naturally contain high amounts of PUFAs and limited antioxidant defences that subsequently

lead to LPO that can impair sperm function [37]. Increased LPO and altered membranes can affect sperm function through impaired metabolism, DNA-damage and increase morphological abnormalities and furthermore decrease caudal sperm motility [38,39].

The most common forms of DM are Type 1 DM and Type 2 DM. Type 1 DM is caused by the destruction of the  $\beta$ -cells in the Islet of Langerhans in the pancreas, which is responsible for insulin production. Lack of insulin production results in glucose transporters, e.g. Glut 4 not being stimulated and thereby glucose cannot enter into the cells. Type 2 DM is caused by insulin resistance, this is when insulin receptors do not respond to insulin stimulation and therefore similarly to Type 1 DM does not allow translocation of the glucose transporters (Glut 5) to the cell surface to allow an influx of glucose with its gradient into the cell to be used for as energy. The breakdown of these macromolecules leads to rapid loss of muscle mass and body weight [40]. The weight loss results in decreased testosterone and estradiol production can be contributed to a decrease in dehydroepiandrosterone (DHEA), which is a precursor of sex steroid hormones, and the enzymes (17 $\beta$ -hydroxysteroid dehydrogenase, 3 $\beta$ -HSD and 5 $\alpha$ -aromatase) responsible for DHEAs conversion to testosterone and estradiol [41]. DHEA is mainly produced by skeletal muscles. Testosterone is responsible for regulating and maintaining growth and proper functioning of the reproductive organs [42,43]. Testicular size has been directly linked to spermatogenesis and sperm quality [44,45]. In this study it was observed that there was a significant decrease in body weights after the 6 week period in the diabetic control group when compared to the normal control group as well as when compared to their initial weights. Epididymal and testicular weights were also decreased after the 6 week period when the diabetic control group was compared to the normal control group, in these animals which in all likelihood could be contributed to decrease levels of testosterone (Table 1).

However, a daily supplementation of *H. hemerocallidea* was able to decrease blood glucose levels and LPO activity. It was also observed that *H. hemerocallidea* supplementation (800 mg/kg) normalised body weights as well as both sperm motility and morphology of diabetic rats. Antioxidant activity of SOD, CAT and GSht as well as the levels plasma levels of testosterone and estradiol was also significantly increased in the diabetic groups treated with both 800 mg/kg and 200 mg/kg of *H. hemerocallidea*, respectively. Previous studies have also shown a positive correlation between supplementation of natural antioxidants and increased testicular SOD and CAT activity as well as GSht levels. For example, Fatani and co-workers [46] demonstrated the effects of lutein dietary supplementation attenuating STZ-induced diabetes and resulted in increased SOD and CAT activity and levels of GSht was also increased. Another study done by Nelli and co-workers [43] showed that  $\alpha$ -mangostin (a plant extract from *Garcinia mangostana* L, Guttiferaceae) significantly increased testicular SOD and CAT activity as well as GSht levels when a STZ-induced diabetic group treated with different doses of  $\alpha$ -mangostin was compared to a diabetic control group. These studies confirmed that botanical sources help stimulate the activity of SOD and CAT as well as GSht levels which may ameliorate diabetes induced male infertility. Nair and co-workers [47] compared *H. hemerocallidea*, Hypoxoside (a compound extracted from *H. hemerocallidea*) as well as rooperol (which is hypoxoside's aglucon) to quercetin and concluded that *H. hemerocallidea* could have value as an antioxidant prodrug. After the 6 week period of treatment there was also a significant improvement on testosterone and estradiol levels. No studies have been done on *H. hemerocallidea* and its effects on sex hormone levels in diabetic rats. From our results

we can state that at a dose of 800 mg/kg *H. hemerocallidea* ameliorated production of both testosterone and estradiol levels in diabetic Wistar rats. Testosterone and estradiol concentrations can also be linked to improvement in both epididymal and testicular weights as both these hormones play a vital role in the growth and functioning of the reproductive organs (Table 4). These results can also be linked to the improved sperm motility and morphology as both sex hormones play a vital role in spermatogenesis (Table 2).

Numerous research studies have measured TAC using FRAP and ORAC assays in liver, kidney, blood plasma and serum. However, not much TAC studies has been performed on epididymal and testicular tissue and therefore it is important to point out that this is the first study measuring the TAC of *H. hemerocallidea* in epididymal and testicular tissue of diabetic rats. ORAC results rely on the amount of damage caused to the fluorescent probe by free radicals. The current study showed a significant decrease in epididymal and testicular FRAP and epididymal ORAC when the diabetic group was compared to the diabetic groups treated with both 800 mg/kg and 200 mg/kg *H. hemerocallidea* respectively. However, testicular ORAC showed no significant difference when the diabetic control group was compared to the *H. hemerocallidea* treated (800 mg/kg and 200 mg/kg) diabetic groups.

## Conclusion

In our study it was observed that the diabetic model was successfully created with a single intraperitoneal injection of STZ as confirmed by the increased blood glucose levels after 3 days. The STZ-induced DM showed to negatively affect (lowered) body-, epididymal- and testicular weights, SOD, CAT, GSht levels, FRAP, ORAC as well as testosterone and estradiol levels. Our findings showed that *H. hemerocallidea* improved antioxidant enzyme activities in normal and under OS conditions, serving as protective mechanism against OS in the reproductive system of male Wistar rats. *H. hemerocallidea* could have further protective effects as was observed due to lowering of blood glucose levels and LPO activity. It was observed that *H. hemerocallidea* has a potential beneficial effect on antioxidant enzyme activities, sperm motility, sperm morphology and serum testosterone and estradiol levels. There was also an improvement in serum testosterone and estradiol levels in the non-diabetic group supplemented with 800 mg/kg *H. hemerocallidea*. Most of the results obtained in this study showed that improvement was not dosage dependent. However, sperm motility and morphology showed the greatest improvement in the diabetic group treated with 800 mg/kg *H. hemerocallidea*. According to these findings we conclude that *H. hemerocallidea* supplementation is an effective approach to ameliorate male infertility in diabetic rats and therefore warrants further investigation in human subjects.

## Limitations of the study

Only two concentrations of *H. hemerocallidea* were tested. The ultimate test would be the effects of *H. hemerocallidea* on diabetic male Wistar fecundity rate. We are planning a follow up study to allow mating and observe its effect on pregnancy outcome.

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