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RESEARCH

# Histomorphological response of D-ribose L-cysteine to ketamine-induced testicular toxicity in adult male Wistar rats

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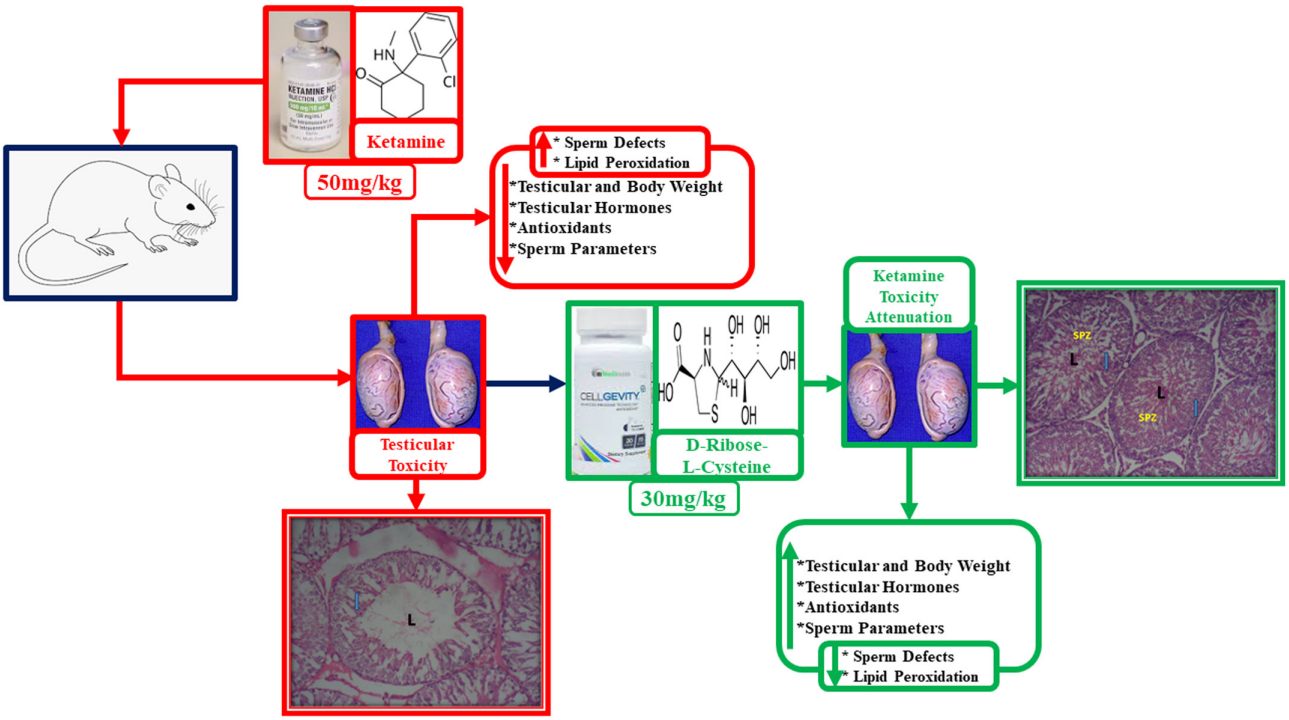
## Graphical Abstract




## Abstract

*Background:* This study aims to study the histomorphological response of D-ribose-L-cysteine (DRLC) to ketamine-induced testicular damage in adult male Wistar rats.
*Methods:* A total of 20 adult male Wistar rats were used for this experiment. The animals were randomly divided into four groups (A–D) ($n = 5$). Group A served as the control, receiving distilled water as placebo; animals in group B were administered with 50 mg/kg body weight (bw) of ketamine only; animals in group C were administered with 50 mg/kg bw of ketamine and 30 mg/kg bw of DRLC; animals in group D were administered with 30 mg/kg bw of DRLC only. At the end of the experiment, blood was taken from the heart

**Keywords**

- ketamine
- D-ribose-L-cysteine
- antioxidant
- histomorphology
- testicular toxicity

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via cardiac puncture and stored, semen was collected from the caudal epididymis for immediate sperm analysis, while the testes were excised and preserved for histological examination and biochemical analysis.

*Results:* The results showed abnormalities marked by a significant decrease in the weights, sperm parameters, as well as antioxidants, serum hormonal levels and abnormal testicular microarchitecture in the rats as a result of ketamine treatment. However, DRLC exhibits significant quenching effects and attenuating activities on the ketamine-induced abnormalities by increasing the rats' weights, restoring the sperm parameters, as well as increasing the antioxidants and serum hormonal levels with restored testicular histoarchitecture.

*Conclusion:* DRLC in the current study attenuated the toxic effects of ketamine on the testes; therefore, it could be used as adjuvant therapy for reproductive toxicant-induced testicular toxicity due to its potent antioxidant property.

**Significance statement**

The testis is a vital secreting organ that produces and stores spermatozoa and is crucial for producing male sexual hormones and is thus the main target of infertility when overdoses of chemicals and toxins are introduced to it. In view of the facts above, studies of the potential of chemicals like ketamine to induce testicular toxicity are important as well as the methods aimed at mitigating this effect. Various studies have been conducted on the effectiveness of DRLC in subsiding different chronic health conditions, but there is no published literature on the effects of DRLC in ketamine-induced testicular toxicity in adult male Wistar rats. Hence we present this study.

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## Introduction

Ketamine is a promising agent for pain and treatment-resistant depression (Zhang & Hashimoto 2019). It is favored over other anesthesia as a non-respiratory and non-cardiac depressant (Gales & Maxwell 2018). In low- and middle-income countries, it is widely used as an anesthetic because it requires less post-operational equipment. It is widely used in veterinary practice to treat large and small domestic and wildlife animal depressants (Gales & Maxwell 2018). The effect of ketamine on the respiratory and circulatory systems is different from that of other anesthetics. When used at anesthetic doses, ketamine usually stimulates rather than depresses the circulatory system. Low blood pressure is harmful in people with a severe head injury, and ketamine is least likely to cause low blood pressure and often even able to prevent it (Hemmingsen & Nielsen 1991). However, the antidepressant action of a single administration of ketamine wanes with time, and the effects of repeated use have not been sufficiently studied (Bobo *et al.* 2020). In comparison to other traditional antidepressants, ketamine has a rapid onset of action (Marcantoni *et al.* 2020). As an anticonvulsant, a further therapeutic application of ketamine is mainly used for the management of refractive epileptic status (Fang & Wang 2015). Ketamine in humans can give hallucinations and is well known as an addiction drug (Fang & Wang 2015). In addition, ketamine has the potential to cause lower urinary tract disease, dysuria, polyuria, hematuria, nightmare, declining sperm quality and central nervous system toxicity (Shamsi *et al.* 2009) Increased misuse of ketamine has raised concerns about its toxic consequences, which may be severe and possibly long-lasting (Shahani *et al.* 2007). The increase in ketamine concentration within the body causes a buildup of reactive oxygen species (ROS) in the cells, resulting in a destructive balance in the free radicals and antioxidants of the body (Maretta 2014). ROS are oxygen averagely reactive species that increase dramatically during periods of environmental stress (ultraviolet ray or heat exposure) and result in significant harm to cell structures (Dubrovsky 2005). Oxidative stress is associated with increased oxidizing agent development or a significant decline in antioxidant resistance efficacy, such as glutathione (GSH) (Falana *et al.* 2017). Oxidative stress factors are implicated in testicular dysfunctions induced by xenobiotics, which contribute to male infertility, but antioxidants act as a defense system, disarming free radicals.

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D-Ribose-L-cysteine (DRLC) is a unique molecule that combines ribose and L-cysteine and, in turn, supplies the body cells with ATP and also delivers cysteine to the cell which enhances GSH (Falana *et al.* 2017). The ribose sugar involved in this combination plays an essential role when DRLC is administered orally (Falana *et al.* 2017). It protects L-cysteine from the effects of the enzymes of metabolism and ensures the absorption of cysteine (a building block of GSH) at the intestines into the bloodstream from where cysteine and ribose reach body cells (Nagasawa 2015). D-Ribose is an L-cysteine medicinal product believed to assist intracellular GSH elevation (Nagasawa 2015). For the general biosynthesis of GSH, L-cysteine acts as a rate-restricting substrate (Vasdev *et al.* 2009), and several cysteine analogs for improving its delivery into cells have been created. DRLC is an analog and pro-drug cysteine produced to enhance the synthesis of GSH (Roberts *et al.* 1987, Lu 2013). It is a dietary supplement designed to supply cysteine to cells, increasing the levels of cell GSH (Roberts *et al.* 1987, Ojetola *et al.* 2021). GSH is present all over the body and has antioxidant roles; however, due to oxidative stress, this can be easily depleted. The GSH stores can result in hypertension and cardiovascular diseases when there is a presence of oxidative stress (Vaziri *et al.* 2000). DRLC is an antioxidant synthesis used to support the production of on-demand GSH by cells (Chandra *et al.* 2015). The whole intake of GSH cannot be successful as it is lost before it reaches the cell during the digestive phase (Roberts *et al.* 1987). These challenges can be resolved through the riboceine portion by guarding and delivering a fragile cysteine compound that allows GSH to be produced by the cells when they are most needed (Vasdev *et al.* 2009). This study, however, was designed to investigate the response of DRLC to ketamine-induced testicular toxicity in adult male Wistar rats.

## Materials and methods

### Chemicals

Ketamine was procured from Sigma Company, and DRLC was procured from Max International (Salt Lake, UT, USA). All other chemicals used in the study were of analytical reagent grade.

### Animals

In this prospective cohort study, a total of 20 adult male Wistar rats weighing 150–200 g and aged 8–10 weeks (*Rattus norvegicus*) were obtained from the animal house, Department of Human Anatomy, Federal University of Technology, Akure. The rats were collected in isolated cages in the experimental house of the Department of Human Anatomy, College of Health Science, Federal University of Technology, Akure. They were maintained with a constant 12 h/12 h darkness and light cycle. All animal handling procedures were approved by the Ethics Committee of the Federal University of Technology, Akure (CHS/FUA/2021/033).

### Experimental protocol

The rats were divided into four groups ($n=5$), labeled as groups A, B, C and D.

Ketamine administration in this study was done according to the procedure described by Cui *et al.* (2019). DRLC was administered using the procedure described by Adelakun *et al.* (2021).

(i) Group A animals represent control and received water as placebo.
(ii) Group B animals were administered 50 mg/kg body weight (bw) of ketamine
(iii) Group C animals were administered 50 mg/kg bw of ketamine and 30 mg/kg bw of DRLC.
(iv) Group D animals were administered 30 mg/kg bw of DRLC only.

All administrations were done orally via an oral cannula, and all animals were observed for any behavioral anomalies, illness and physical anomalies. The experimental procedures were in accordance with the provided recommendations in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Sciences. The rats were fed with a standard rat chow diet, and drinking water was supplied *ad libitum*. The weights of the animals were recorded at procurement, during acclimatization, at the commencement of the experiment and weekly throughout the experimental period using a CAMRY electronic scale (EK5055, Indian).

### Surgical procedure

After the last administration, the rats were administered intraperitoneal pentobarbital sodium (40 mg/kg), and their abdominal region was opened and the testes of all the animals were immediately removed. The testicular weights of each rat were recorded. The rats were decapitated, and blood samples were collected for analysis. The blood samples were centrifuged at 4°C for 10 min at 250 ***g***, and the serum obtained was stored at –20°C until assayed. The harvested testis specimens were fixed in Bouin's fluid for histological analysis (Avwioro 2010).

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### Epididymis sperm count, viability and motility

The spermatozoa were obtained from the cauda epididymis by cutting into 2 mL of medium (Hams F10) containing 0.5% bovine serum albumin (Feng *et al.* 2001). After 5 min of incubation at 37°C (with 5% $CO_2$), the cauda epididymis sperm reserves were determined using a hemocytometer. Sperm motility was analyzed with a microscope (Leica DM750) and reported as the mean number of motile sperm according to the method developed by the World Health Organization (WHO 1999).

### Lipid peroxidation (malondialdehyde) and antioxidant markers (GSH, SOD and catalase)

The level of lipid peroxidation products in the rat testes marked by malondialdehyde (MDA) was estimated following the method published by Niehaus and Samuelsson (1968). Nonenzymatic antioxidants in the rat testes marked by reduced GSH and enzymatic antioxidant marker catalase (CAT) were estimated as described by Ellman (1959) and Sinha (1972), respectively. Superoxide dismutase (SOD) activity in the testes was determined according to the method described by Marklund and Marklund (1974).

### Hormone determination

The hormonal levels of testosterone (TT), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured using available immunoassay (ELISA) kits (Randox Laboratories Ltd, Ardmore Diamond Road, Crumlin County, Antrim, UK Qt94QY) according to manufacturer's instructions, following the method of Ogunlade *et al.* (2021).

### Testicular histology preparation

The testes of the rats were harvested and fixed in Bouin's fluid for 24 h before being transferred to 70% alcohol for dehydration. The tissues were passed through 90% and absolute alcohol and xylene for different durations before being transferred into molten paraffin wax for 1 h each in an oven at 65°C for infiltration. The tissues were embedded, and serial sections cut on a rotary microtome set at 5 μm were performed. The tissues were picked up with albumenized slides and allowed to dry on hot plates for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (two changes), 70% alcohol, 50% alcohol (in that order) and then in water for 5 min. The slides were then stained with hematoxylin and eosin, mounted in dibutylphthalate polystyrene xylene (DPX), and photomicrographs were taken at a magnification of 100× on a Leica DM750 microscope (Adelakun *et al.* 2019).

### Statistical analysis

The data obtained were analyzed statistically using one-way ANOVA, followed by Dunnett's comparison test. Data were expressed as mean ± S.E.M.. The level of significance was $P < 0.05$. Data were analyzed using GraphPad Prism 5 Windows (GraphPad Software).

## Results

### Full protective effect of DRLC against the decrease of rat body weight induced by ketamine treatment

The result revealed that rats treated with Kketamine only (group B) showed a significant decrease ($P < 0.05$) in bw when compared with the control, ketamine and DRLC (group C) and DRLC-only (group D) groups (Fig. 1). However, there was no significant difference in the bw of the animals administered with ketamine and DRLC and DRLC-only groups when compared with the control (Fig. 1).

### Full protective effect of DRLC against the decrease of rat testis weight (right and left) provoked by ketamine treatment

The result revealed that rats treated with ketamine only (group B) showed a significant decrease ($P < 0.05$) in testis weight (both left and right) when compared with the control, ketamine and DRLC (group C) and DRLC-

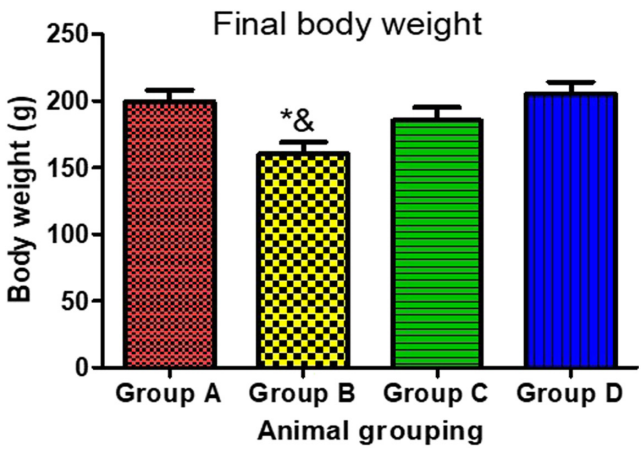


**Figure 1**
Effect of DRLC on the body weight on ketamine-induced control and experimental rats. *$P < 0.05$ as compared to group A; & $P < 0.05$ as compared to groups C and D.

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only (group D) groups (Fig. 2). However, there was no significant difference in the testis weights (both left and right) of the animals administered with ketamine and DRLC and DRLC-only groups when compared with the control (Fig. 2).

## Changes in sperm morphology exerted by ketamine treatment: significant counteracting effect by DRLC

The result revealed that rats treated with ketamine only showed a significant increase ($P < 0.05$) in defective sperm morphology neck and tail defects (ND and TD) but no significant difference in head defect (HD) compared to the control (Fig. 3). However, the rats administered with both ketamine and DRLC and DRLC only showed a significant increase ($P < 0.05$) in sperm morphology (normal) and decrease in sperm morphological defects (ND and TD) but no significant difference in HD when compared with the ketamine-only group (group C and D vs group B) (Fig. 3). Also, the rats administered with ketamine and DRLC only showed a significant increase ($P < 0.05$) in defective sperm morphology (ND and TD) but no significant difference in HD when compared with ketamine-only (group A) and DRLC-only (group D) groups (Fig. 3).

## Significant quenching effect of DRLC treatment on the reduction of sperm motility, concentration count, volume and viability provoked by ketamine

The results revealed that rats treated with ketamine only showed a significant decline ($P < 0.05$) in sperm motility, concentration count, semen volume and sperm viability relative to the control group (Fig. 4). The rats administered

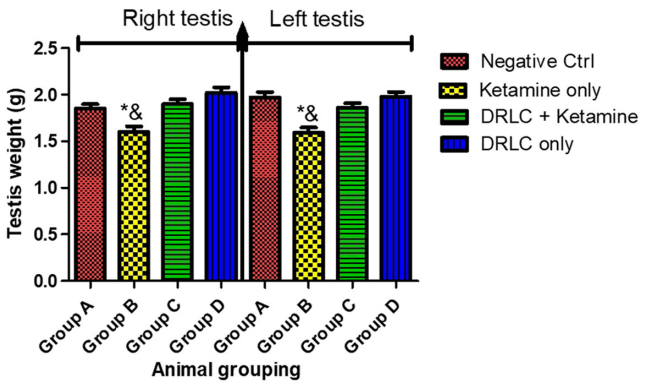


**Figure 2**
Effect of DRLC on testis weight (right and left) on ketamine-induced control and experimental rats. *$P < 0.05$ as compared to group A; &$P <$ 0.05 as compared to groups C and D.

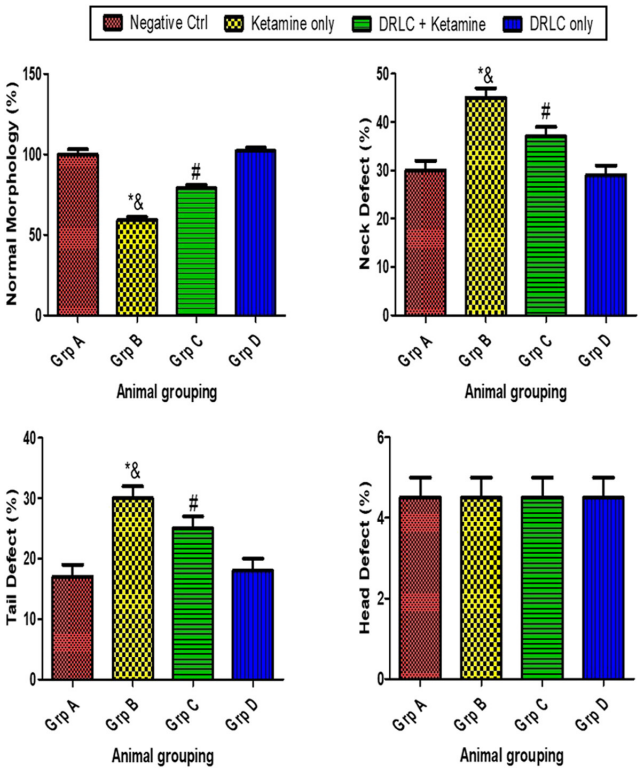


**Figure 3**
Effect of DRLC on sperm morphology (ND, TD, HD and normal) on ketamine-induced control and experimental rats. *$P < 0.05$ as compared to groups A and D; &$P < 0.05$ as compared to group C; #$P < 0.05$ as compared to groups A and D.

with both ketamine and DRLC showed a significant increase ($P < 0.05$) in sperm motility, concentration count, semen volume and sperm viability when compared with the ketamine-only group (Fig. 4). However, there was a significant decrease in these characteristics when the rats administered with both ketamine and DRLC were compared with the control and DRLC-only group ($P < 0.05$) (Fig. 4). However, there was no significant difference between the control and DRLC-only group when they were compared with one another.

## Significant quenching effect of DRLC treatment on the MDA increase and the GSH, SOD and CAT decrease in the testes of rats challenged with ketamine

The results revealed there was a significant decrease in CAT, SOD and GSH levels but a significant increase in MDA level among the animals treated with ketamine only (group B) when compared to the control group (group A) ($P < 0.05$) (Fig. 5). However, there was an increase in CAT, SOD and GSH levels and a decrease in MDA level among the animals that received combined administration of

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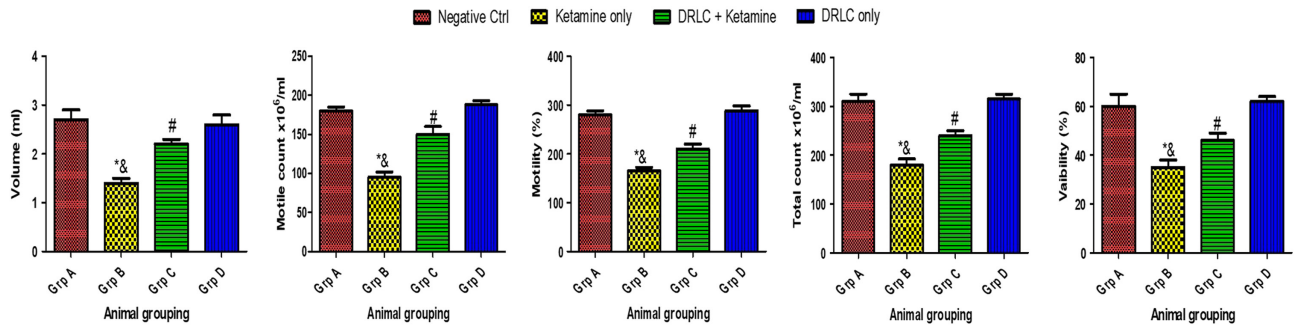


**Figure 4**
Effect of DRLC on sperm motility, concentration count, volume and viability on ketamine-induced control and experimental rats. *$P$ < 0.05 as compared to groups A and D; &$P$ < 0.05 as compared to group C; #$P$ < 0.05 as compared to groups A and D.

ketamine and DRLC and DRLC only (groups C and D) compared with animals treated with ketamine only (group B) ($P$ < 0.05) (Fig. 5). There was a significant decrease in CAT, SOD and GSH serum levels and an increase in MDA serum level among the animals that received combined administration of ketamine and DRLC when compared with the control (group A) and DRLC-only group (group D) (Fig. 5). There was no significant difference in CAT, SOD, GSH and MDA levels when the control (group A) and animals that received DRLC only (group D) were compared with each other.

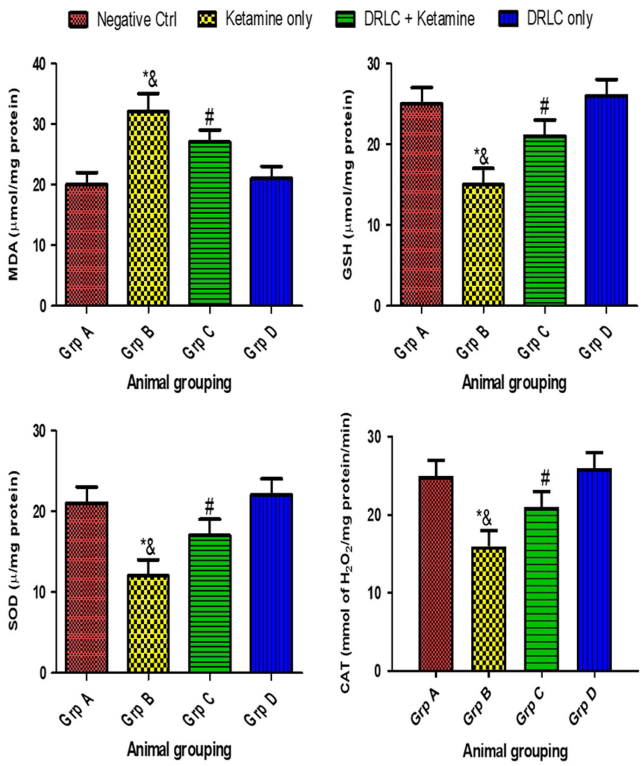


**Figure 5**
Effect of DRLC on MDA, GSH, SOD and CAT levels on ketamine-induced control and experimental rats. *$P$ < 0.05 as compared to groups A and D; &$P$ < 0.05 as compared to group C; #$P$ < 0.05 as compared to groups A and D.

## Significant quenching effect of DRLC on the net decrease of FSH, LH and testosterone serum levels provoked by ketamine treatment

There was a significant decrease in FSH, LH and testosterone serum levels in animals that were treated with ketamine only (group B) when compared to the control group (group A) ($P$ < 0.05) (Fig. 6). There was a significant increase in FSH, LH and testosterone serum levels among the animals that received combined administration of ketamine and DRLC and DRLC only (groups C and D) compared with animals treated with ketamine only (group B) ($P$ < 0.05) (Fig. 6). However, there was a significant decrease in FSH, LH and testosterone serum levels among the animals that received combined administration of ketamine and DRLC when compared with the control (group A) and DRLC-only group (group D) (Fig. 6). There was no significant difference in FSH, LH and testosterone serum levels when the control (group A) and animals that received DRLC only (group D) were compared with each other.

## Testicular photomicrographs showing a full protective effect of DRLC treatment against the derangement of the seminiferous epithelium as determined by the ketamine challenge

The histological observation showed a normal testicular microarchitecture with a stratified seminiferous epithelium that is oval in shape and a lumen holding numerous spermatogenic cells with prominent Leydig cells and abundant spermatozoa in the control group (Fig. 7A). However, in the ketamine-only group, an abnormal testicular microarchitecture was observed which is characterized with disruption of spermatogenesis and

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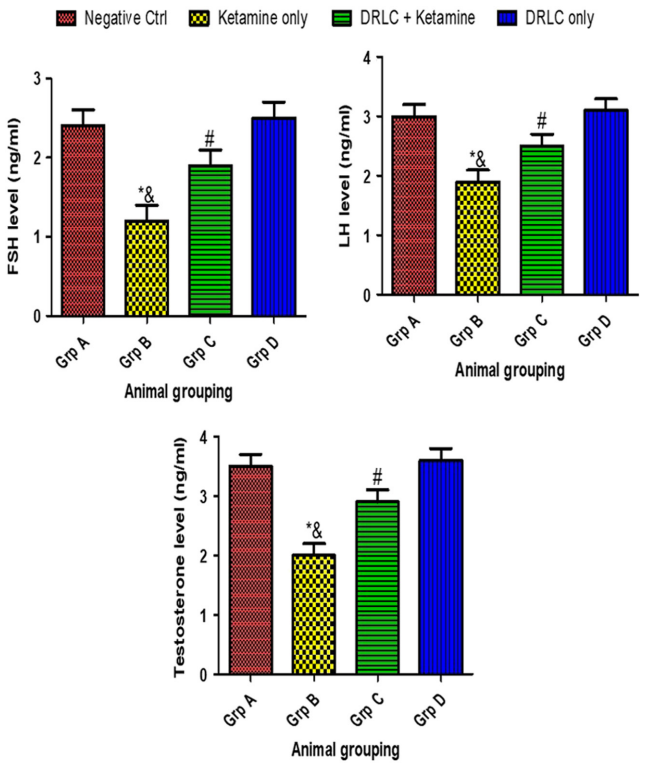


**Figure 6**
Effect of DRLC on serum level of FSH, LH and testosterone on ketamine-induced control and experimental rats. $^{*}P < 0.05$ as compared to groups A and D; $^{\&}P < 0.05$ as compared to group C; $^{\#}P < 0.05$ as compared to groups A and D.

empty lumen as well as a distorted seminiferous tubule and Leydig cells with no spermatozoa on sight (Fig. 7B). Ketamine-and-DRLC-treated animals together with the DRLC-only-treated animals showed visible spermatozoa and a completely restored lumen and spermatogenic cells similar to that of the control (Fig. 7C and 7D).

## Discussion

Ketamine is a frequently overused anesthetic that is mostly abused by young people during parties and clubs (Hsu *et al.* 2014, Lu *et al.* 2016). This dissociative anesthetic substance is now one of the most often abused drugs on the planet. The testis is considered the most important organ in the male reproductive system (Wang *et al.* 2017). It has two main functions: steroid hormone synthesis and sperm production. Various factors affect spermatogenesis, including medicines and toxic elements in environmental pollution (Jenardhanan *et al.* 2016). Oxidative stress reflects an imbalance between the systemic manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or repair the resulting damage

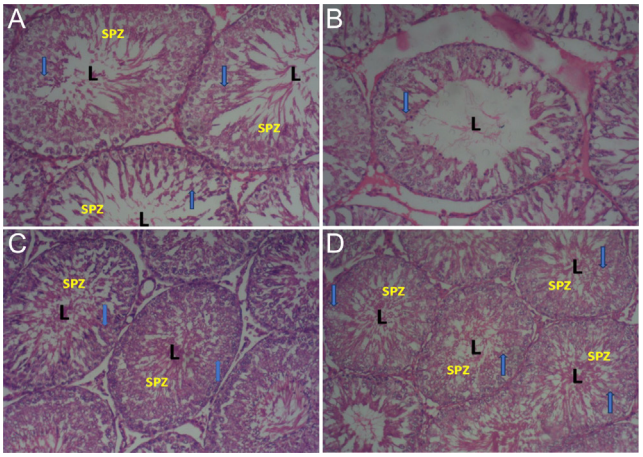


**Figure 7**
Testicular photomicrographs showing the effects of DRLC on ketamine-induced control and experimental rats. A: control group (group A); B: ketamine-only-treated group (group B); C: ketamine- and-DRLC-treated group (group C); D: DRLC-only group (group D) SPZ, spermatozoa; L, lumen; arrow: spermatogenic cells. Stains: hematoxylin and eosin. Magnification ×100.

(Falana *et al.* 2017). Thus, this causes disruptions in the normal mechanisms of cellular signaling and cell death (Chandra *et al.* 2015). One of the biological systems (antioxidants) that detoxify ROS or repair the resulting damages caused by free radicals is GSH. Sometimes, these free radicals overpower the biological systems; thus, the body may need external supplements to complement the production of antioxidants (Chandra *et al.* 2017). DRLC is capable of boosting male fertility because of its potent antioxidant properties and its great abundance of GSH (Adelakun *et al.* 2021). This present study evaluates the histopathological response of DRLC on ketamine-induced testicular toxicity in adult male Wistar rats.

The weights of the testes and accessory sexual organs reflect the androgenic status of the animal. Products of normal spermatogenesis and fluids excreted by Sertoli cells are the main contributors to testicular weight (Biswas *et al.* 2001, Aly & Hassan 2018). The result from this study revealed that the rats administered with ketamine only showed a significant reduction in both the testicular and bw when compared with the control. The reduction in the bw and testicular weight following ketamine administration is consistent with a study by Qi *et al.* (2017) who reported a drastic reduction in the testicular and bw of the rats following a chronic administration of ketamine for 6 weeks. However, the rats treated with DRLC following ketamine administration showed a significant improvement in the testicular and bw when compared with those administered with ketamine only. This can be attributed to the DRLC's ability to reverse reproductive

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dysfunctions, as reported by Wankeu-Nya *et al.* (2013) and Adelakun *et al.* (2018).

Sperm morphology displays a potential impact on sperm function and may ultimately impact reproductive ability (De Braekeleer *et al.* 2015). From this study, it was also discovered that the rats administered with ketamine only showed a significant increase in defective sperm morphology characterized by sperm HD, ND and TD when compared to the control that shows a normal sperm morphology. This is consistent with a report by Absalan *et al.* (2014) which reported an increased defective sperm morphology following ketamine administration in rat models. However, the rats treated with DRLC following ketamine administration showed a significant increase in normal sperm morphology and a decrease in defective sperm morphology when compared with those administered with ketamine only. This may be attributed to the anti-mutagenic effects of DRLC (Falana *et al.* 2017).

Sperm concentration, motility and normalcy have been proposed as the three key parameters in evaluating infertility (Pasqualotto *et al.* 2000). The result from this study showed that the rats administered with ketamine only showed a significant reduction in sperm motility, concentration count, semen volume and sperm viability when compared with the control. Although the mechanism of ketamine effects on sperm parameters is unclear, several clinical models suggest a link between sperm parameters and an increase in ROS. Pasqualotto *et al.* (2000) showed that an increase in ROS in semen can cause a negative effect on semen parameters. However, the rats treated with DRLC following ketamine administration showed a significant increase in sperm motility, concentration count, semen volume and sperm viability when compared with those administered with ketamine only. This can be attributed to the antioxidative properties of DRLC. Researchers have shown that antioxidant levels in the blood correlated with sperm counts and motility (Shamsi *et al.* 2009, 2010).

Oxidative stress represents an imbalance between the production of ROS or free radicals and the available antioxidant system (Agarwal & Prabakaran 2005). ROS, on the other hand, has been linked to reduced male fertility due to peroxidative damage to the sperm plasma membrane (Kodama *et al.* 1997). The result from this study showed that the rats administered with ketamine only showed a significant increase in MDA level and a corresponding decrease in CAT, SOD and GSH levels when compared with the control. This result is concurrent with a study by Giwercman *et al.* (2003) that reported the link between ketamine, antioxidant levels and lipid peroxidation on testicular integrity. However, the rats treated with DRLC following ketamine administration showed a significant decrease in MDA level as well as a corresponding increase in CAT, SOD and GSH levels when compared with those administered with ketamine only. This may be due to the antioxidative protective effects of D-ribose and L-cysteine. The presence of cysteine in the body's cells enables the production of GSH, while ribose produces cellular energy (Falana *et al.* 2017). It is the interaction between D-ribose and L-cysteine that facilitates the production of GSH. The GSH within the cell protects against destruction from free-radical damage (Adelakun *et al.* 2021).

In both males and females, gametogenesis is regulated by the hypothalamic–pituitary–gonadal axis that corresponds to the hormonal axis, gonadotropin-releasing hormone (GnRH) and gonadotropin steroids (Qi *et al.* 2017). The main target of GnRH is the gonadotrope cells, located in the adenohypophysis. These in return release two gonadotropin hormones, FSH and LH, which through the main circulation reach the gonads to regulate gametogenesis via the synthesis of steroid hormones (Qi *et al.* 2017). In the male, LH stimulates the production of testosterone by testicular Leydig cells. FSH binds to receptors on the surface of Sertoli cells and functions in concert with testosterone to promote the proliferation of spermatogonia as well as the meiosis and post-meiotic development of germ cells (Ding *et al.* 2015). From this study, there was a significant decrease in FSH, LH and testosterone serum levels in animals that were administered with ketamine only when compared to the control group. Ketamine has been said to influence the gonadotropic hormones FSH and LH as well as prolactin and testosterone (Araghi *et al.* 2017). The effect of ketamine on FSH, LH and testosterone may be attributed to the direct depressant action of ketamine on the pituitary gland (Qi *et al.* 2017). However, the rats treated with DRLC following ketamine administration showed a significant increase in FSH, LH and testosterone serum levels when compared with those administered with ketamine only. The increased serum hormonal levels can be attributed to enhanced production of FSH and LH by the anterior pituitary gland which promotes follicle development and testosterone synthesis by DRLC (Jiang *et al.* 2018).

Histopathological examination of the testes provides valuable information regarding the assessment of the severity of toxicity, the cellular site of damage and the possibility of recovery (Uygur *et al.* 2014). Apoptosis, necrosis and autophagy are the main three pathways

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of cells to die. Apoptosis is a genetically programmed pathway that results from external and/or internal factors that end with cell death (Grilo & Mantalaris 2019). From this study, an abnormal testicular microarchitecture was observed in the animals administered with ketamine only when compared with the control that showed normal testicular microarchitecture with an oval-shaped stratified seminiferous epithelium and a lumen holding numerous spermatogenic cells with prominent Leydig cells and abundant spermatozoa. This result indicates that ketamine increased apoptosis in testicular tissues, which is in line with the results of other studies, which showed that ketamine evoked apoptosis in other tissues, such as hepatic cells, the brain and the urinary bladder (Kalkan *et al.* 2014, Liu *et al.* 2015, Pancaro *et al.* 2016). However, the rats treated with DRLC following ketamine administration showed visible spermatozoa and a completely restored lumen and spermatogenic cells similar to that of the control when compared with those administered with ketamine only. This can be attributed to the anti-apoptotic properties of DRLC, as reported by Okoh *et al.* (2020).

## Conclusion

In conclusion, it can be deduced from this study that chronic ketamine treatment has hazardous effects on reproductive functions. Ketamine adversely affects sex hormones and sperm parameters. These effects occur via multiple mechanisms involving lipid peroxidation, apoptosis and Sertoli cell disruption. However, DRLC in the current study attenuated the toxic effects of ketamine on the testes; therefore, it could be used as adjuvant therapy for reproductive toxicant-induced testicular toxicity due to its potent antioxidant property.

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The authors declare that they have no conflicts of interest.

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**Ethics approval**
The experimental procedures were conducted under the NIH guidelines for the care and use of laboratory animals in line with guidelines of the Department of Human Anatomy, College of Health Science, Federal University of Technology, Akure, Nigeria and the Health Research and Ethics Committee of the Federal University of Technology, Akure, Nigeria (CHS/FUA/2021/033).

**Author contribution statement**
Mr A.O.A.: Methodology, Writing – original draft, Formal analysis, Investigation, Graphical abstract; Dr C.C.C.: Methodology, Project administration, Supervision, Investigation; Mr B.A.F.: Writing – original draft, Investigation; Ms O.M.A.: Proofreading and Formatting; Mr B.J.O.: Proofreading and Formatting; Dr O.B.: Conceptualization, Methodology, Validation, Writing – review and editing, Investigation.

## References

Absalan F, Ghannadi A & Zabihi A 2014 The effects of different doses of ketamine on quality of normal ejaculated sperm. *International Journal of Fertility and Sterility* **8** 207–214.

Adelakun SA, Ogunlade B, Fidelis OP & Ajao AA 2021 Nutritional supplementation of D-ribose-L-cysteine suppresses oxidative stress, spermatogenesis and steroidogenesis recovery in rats exposed to mercury chloride: histomorphometry and biochemical evidence. *Endocrine and Metabolic Science* **4** 100–105. (https://doi.org/10.1016/j.endmts.2021.100105)

Adelakun SA, Omotoso OD & Aniah JA 2018 Modulating role of D-ribose-L-cysteine on oxidative stress in streptozotocin-induced diabetes on plasma lipoprotein, oxidative status, spermatogenesis and steroidgenesis in male Wistar rats. *Current Research in Diabetes and Obesity Journal* **9** 1–7.

Adelakun SA, Ukwenya VO, Ogunlade BS, A J & G A 2019 Nitrite-induced testicular toxicity in rats: therapeutic potential of walnut oil. *JBRA Assisted Reproduction* **23** 15–23. (https://doi.org/10.5935/1518-0557.20180062)

Agarwal A & Prabakaran SA 2005 Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. *Indian Journal of Experimental Biology* **43** 963–974.

Aly HAA & Hassan MH 2018 Potential testicular toxicity of gentamicin in adult rats. *Biochemical and Biophysical Research Communications* **497** 362–367. (https://doi.org/10.1016/j.bbrc.2018.02.085)

Araghi A, Tabari A & Golshahi H 2017 Protective effect of carvacrol on ketamine induced testicular damage in mouse model of schizophrenia. *Journal of HerbMed Pharmacology* **6** 100–106.

Avwioro OG 2010 *Histology and Tissue Pathology. Principles and Techniques*. 2nd ed: Ibadan: Claverianum Press.

Biswas NM, Gupta RS, Chattopadhyay A, Choudhury GR & Sarkar M 2001 Effect of atenolol on cadmium-induced testicular toxicity in male rats. *Reproductive Toxicology* **15** 699–704. (https://doi.org/10.1016/s0890-6238(01)00184-8)

Bobo WV, Riva-Posse P, Goes FS & Parikh SV 2020 Next-step treatment considerations for patients with treatment-resistant depression that responds to low-dose intravenous ketamine. *Focus* **18** 181–192. (https://doi.org/10.1176/appi.focus.20190048)

Chandra K, Syed AS, Mohd A, Sweety R & Ali KN 2015 Protection against FCA induced oxidative stress induced DNA damage as a model of arthritis and in vitro anti-arthritic potential of Costus speciosus Rhizome Extract. *International Journal of Pharmacognosy and Phytochemical Research* **7** 383–389.

Cui Y, Hu S & Hu H 2019 Lateral habenular burst firing as a target of the rapid antidepressant effects of ketamine. *Trends in Neurosciences* **42** 179–191. (https://doi.org/10.1016/j.tins.2018.12.002)

De Braekeleer M, Nguyen MH, Morel F & Perrin A 2015 Genetic aspects of monomorphic teratozoospermia: a review. *Journal of Assisted Reproduction and Genetics* **32** 615–623. (https://doi.org/10.1007/s10815-015-0433-2)

Ding Y, Yu J, Qu P, Ma P & Yu Z 2015 The negative effects of chronic exposure to isoflurane on spermatogenesis via breaking the hypothalamus-pituitary-gonadal equilibrium. *Inhalation Toxicology* **27** 621–628. (https://doi.org/10.3109/08958378.2015.1080772)

Dubrovsky BO 2005 Steroids, neuroactive steroids and neurosteroids in psychopathology. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **29** 169–192. (https://doi.org/10.1016/j.pnpbp.2004.11.001)

Ellman GL 1959 Tissue sulphydryl groups. *Archives of Biochemistry and Biophysics* **82** 70–77. (https://doi.org/10.1016/0003-9861(59)90090-6)
Falana B, Adeleke O, Orenolu M, Osinubi A & Oyewopo A 2017 Effect of D-ribose-L-cysteine on aluminum induced testicular damage in male Sprague-Dawley rats. *JBRA Assisted Reproduction* **21** 94–100. (https://doi.org/10.5935/1518-0557.20170023)
Fang Y & Wang X 2015 Ketamine for the treatment of refractory status epilepticus. *Seizure* **30** 14–20. (https://doi.org/10.1016/j.seizure.2015.05.010)
Feng R, He W & Ochi H 2001 A new murine oxidative stress model associated with senescence. *Mechanisms of Ageing and Development* **122** 547–559. (https://doi.org/10.1016/s0047-6374(01)00232-9)
Gales A & Maxwell S 2018: recent evidence and current uses. *ATOTW* **381** 1–7.
Giwercman A, Richthoff J, Hjøllund H, Bonde JP, Jepson K, Frohm B & Spano M 2003 Correlation between sperm motility and sperm chromatine structure assay parameters. *Fertility and Sterility* **80** 1404–1412. (https://doi.org/10.1016/s0015-0282(03)02212-x)
Grilo AL & Mantalaris A 2019 Apoptosis: a mammalian cell bioprocessing perspective. *Biotechnology Advances* **37** 459–475. (https://doi.org/10.1016/j.biotechadv.2019.02.012)
Hemmingsen C & Nielsen JE 1991 Intravenous ketamine for prevention of severe hypotension during spinal anaesthesia. *Acta Anaesthesiologica Scandinavica* **35** 755–757. (https://doi.org/10.1111/j.1399-6576.1991.tb03385.x)
Hsu J, Lin JJ & Tsay WI 2014 Analysis of drug abuse data reported by medical institutions in Taiwan from 2002 to 2011. *Journal of Food and Drug Analysis* **22** 169–177. (https://doi.org/10.1016/j.jfda.2014.01.019)
Jenardhanan P, Panneerselvam M & Mathur PP 2016 Effect of environmental contaminants on spermatogenesis. *Seminars in Cell and Developmental Biology* **59** 126–140. (https://doi.org/10.1016/j.semcdb.2016.03.024)
Jiang X, Chu Q, Li L, Qin L, Hao J, Kou L, Lin F & Wang D 2018 The antifatigue activities of *Tuber melanosporum* in a mouse model. *Experimental and Therapeutic Medicine* **15** 3066–3073. (https://doi.org/10.3892/etm.2018.5793)
Kalkan Y, Tomak Y, Altuner D, Tumkaya L, Bostan H, Yilmaz A, Unal D, Kara A & Turan A 2014 Hepatic effects of ketamine administration for 2 weeks in rats. *Human and Experimental Toxicology* **33** 32–40. (https://doi.org/10.1177/0960327112472990)
Kodama H, Yamaguchi R, Fukuda J, Kasai H & Tanaka T 1997 Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertility and Sterility* **68** 519–524. (https://doi.org/10.1016/s0015-0282(97)00236-7)
Liu KM, Chuang SM & Long CY 2015 Ketamine-induced ulcerative cystitis and bladder apoptosis involve oxidative stress mediated by mitochondria and the endoplasmic reticulum. *American Journal of Physiology. Renal Physiology* **309** 318–331.
Lu SC 2013 Glutathione synthesis. *Biochimica et Biophysica Acta* **1830** 3143–3153. (https://doi.org/10.1016/j.bbagen.2012.09.008)
Lu YY, Lin CH & Lane HY 2016 Mania following ketamine abuse. *Neuropsychiatric Disease and Treatment* **12** 237–239. (https://doi.org/10.2147/NDT.S97696)
Marcantoni WS, Akoumba BS, Wassef M, Mayrand J, Lai H, Richard-Devantoy S & Beauchamp S 2020 A systematic review and meta-analysis of the efficacy of intravenous ketamine infusion for treatment resistant depression: January 2009 –January 2019. *Journal of Affective Disorders* **277** 831–841. (https://doi.org/10.1016/j.jad.2020.09.007)
Maretta M, Marettov E & Legath J 2014 Toxic effects of cadmium on testis of birds and mammals. *Animal Reproduction Science* 1–55.
Marklund S & Marklund G 1974 Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47** 469–474. (https://doi.org/10.1111/j.1432-1033.1974.tb03714.x)
Nagasawa HT 2015 Method to enhance delivery of glutathione and ATP levels in cells. *Google Patents*. US8501700B2.
Niehaus WG Jr & Samuelsson B 1968 Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *European Journal of Biochemistry* **6** 126–130. (https://doi.org/10.1111/j.1432-1033.1968.tb00428.x)
Ogunlade B, Adelakun SA, Ukwenya VO & Elemoso TT 2021 Potentiating response of D- ribose-L-cysteine on sodium arsenate-induced hormonal imbalance, spermatogenesis impairments and histomorphometric alterations in adult male Wistar rat. *JBRA Assisted Reproduction* **25** 358–367. (https://doi.org/10.5935/1518-0557.20200109)
Ojetola AA, Adedeji TG & Fasanmade AA 2021 Changes in antioxidants status, atherogenic index and cardiovascular variables after prolonged doses of D-ribose-L-cysteine in male Wistar rats. *Heliyon* **7** e06287. (https://doi.org/10.1016/j.heliyon.2021.e06287)
Okoh L, Ajayi AM, Ben-Azu B, Akinluyi ET, Emokpae O & Umukoro S 2020 D-ribose-L-cysteine exhibits adaptogenic-like activity through inhibition of oxido-inflammatory responses and increased neuronal caspase-3 activity in mice exposed to unpredictable chronic mild stress. *Molecular Biology Reports* **47** 7709–7722. (https://doi.org/10.1007/s11033-020-05845-1)
Pancaro C, Segal BS, Sikes RW, Almeer Z, Schumann R, Azocar RJ & Marchand JE 2016 Dexmedetomidine and ketamine show distinct patterns of cell degeneration and apoptosis in the developing rat neonatal brain. *Journal of Maternal-Fetal and Neonatal Medicine* **29** 3827–3833. (https://doi.org/10.3109/14767058.2016.1148132)
Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ & Agarwal A 2000 Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertility and Sterility* **73** 459–464. (https://doi.org/10.1016/s0015-0282(99)00567-1)
Qi L, Liu JY, Zhu YL, Liu W, Zhang SD, Liu WB & Jiang JJ 2017 Toxic effects of ketamine on reproductive system via disrupting hypothalamic-pituitary-testicular axis. *European Review for Medical and Pharmacological Sciences* **21** 1967–1973.
Roberts JC, Nagasawa HT, Zera RT, Fricke RF & Goon DJ 1987 Prodrugs of L-cysteine as protective agents against acetaminophen-induced hepatotoxicity. *Journal of Medicinal Chemistry* **30** 91–96.
Shahani R, Streutker C, Dickson B & Stewart RJ 2007 Ketamine-associated ulcerative cystitis: a new clinical entity. *Urology* **69** 810–812. (https://doi.org/10.1016/j.urology.2007.01.038)
Shamsi MB, Venkatesh S, Kumar R, Gupta NP, Malhotra N, Singh N, Mittal S, Arora S, Arya DS, Talwar P, *et al.* 2010 Antioxidant levels in blood and seminal plasma and their impact on sperm parameters in infertile men. *Indian Journal of Biochemistry and Biophysics* **47** 38–43.
Shamsi MB, Venkatesh S, Tanwar M, Talwar P, Sharma RK & Dhawan A 2009 DNA integrity and semen quality 22 in men with low seminal antioxidant levels. *Mutation Research* **665** 29–36.
Sinha AK 1972 Colorimetric assay of catalase. *Analytical Biochemistry* **47** 389–394. (https://doi.org/10.1016/0003-2697(72)90132-7)
Uygur R, Aktas C, Tulubas F, Uygur E, Kanter M, Erboga M, Caglar V, Topcu B & Ozen OA 2014 Protective effects of fish omega-3 fatty acids on doxorubicin-induced testicular apoptosis and oxidative damage in rats. *Andrologia* **46** 917–926. (https://doi.org/10.1111/and.12173)
Vasdev S, Singal P & Gill V 2009 The antihypertensive effect of cysteine. *International Journal of Angiology* **18** 7–21. (https://doi.org/10.1055/s-0031-1278316)
Vaziri ND, Wang XQ, Oveisi F & Rad B 2000 Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension* **36** 142–146. (https://doi.org/10.1161/01.hyp.36.1.142)
Wang JW, Kivovich V & Gordon L 2017 Ketamine abuse syndrome: hepatobiliary and urinary pathology among adolescents in Flushing,

NY. *Pediatric Emergency Care* **33** e24–e26. (https://doi.org/10.1097/PEC.0000000000000502)

Wankeu-Nya M, Florea A, Bâlici S, Watcho P, Matei H & Kamanyi A 2013 Dracaena arborea alleviates ultra-structural spermatogenic alterations in streptozotocin-induced diabetic rats. *BMC Complementary and Alternative Medicine* **13** 71. (https://doi.org/10.1186/1472-6882-13-71)

WHO-World Health Organization 1999 *WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction*. 4th ed: Cambridge: University Press.

Zhang K & Hashimoto K 2019 An update on ketamine and its two enantiomers as rapid-acting antidepressants. *Expert Review of Neurotherapeutics* **19** 83–92. (https://doi.org/10.1080/14737175.2019.1554434)

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