Journal of Sustainable Veterinary & Allied Sciences
P-ISSN-2695-2661 e-ISSN: 2811-1346
JoSVAS 2023 December Vol 5 Issue 2: 80-85

Original Research

Effects of Vitamin C on reproductive parameters of rabbit bucks with experimentally induced metronidazole toxicity

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ABSTRACT

This study evaluated the effect of Vitamin C on the reproductive parameters of rabbit bucks experimentally exposed to metronidazole. Twenty (20) adult rabbit bucks were used for the study. The animals were grouped into four groups of five animals each as follows; Group I are the untreated control, group II were administered metronidazole 400mg/kg/day for 30 days, group III were administered 400mg/kg/day of metronidazole for 30days and Vitamin C 200mg/kg/day for 30days and group IV were administered Vitamin C 200mg/kg/day for 30days. The body weight of the animals was taken before and after the experiment. Semen samples were collected from each buck through the use of an improvised artificial vagina (AV) and analysed using standard procedure. The testes and epididymis from three of the rabbits in each group were used for sperm reserve analyses. The obtained data were analysed with Graphpad Prism version 5.0.3 and presented as Mean ± SEM using ANOVA. Values of $P \leq 0.05$ were considered significant. The study found no significant ($P > 0.05$) difference in the body weight of the bucks but found significant ($P < 0.05$) difference in the testicular and epididymal weight, sperm motility, spermatozoz concentration, percentage sperm livability, percentage sperm abnormalities and gonadal and epididymal sperm reserve of rabbit bucks exposed to metronidazole which were reversed by vitamin C. Rabbit bucks showed reproductive damage at therapeutic metronidazole doses and the effects improved with vitamin C administration. Antioxidants (Vitamin C) may be clinically relevant in reproductive toxicity.

Keywords: Epididymis, metronidazole, spermatozoa, testes, Vitamin C

INTRODUCTION

Various factors including drug treatment, toxins, and environmental pollutants have been found to have detrimental impacts on animal reproduction (El-Sayed *et al.*, 2016). Metronidazole is a frequently employed antimicrobial agent utilised in the management of diverse bacterial and parasitic infections (Raza *et al.*, 2018). In spite of its extensive therapeutic usage, metronidazole has been demonstrated to elicit detrimental effects on the reproductive system of male animals, resulting in reduced testis weight and diminished serum testosterone levels (Chauhan *et al.*, 2016). Due to the reported effect of metronidazole on the reproductive system it is important to study the mechanisms that contribute to the reproductive toxicity of metronidazole and to identify potential preventive or therapeutic interventions due to its detrimental effects on the reproductive system (Chauhan *et al.*, 2016). According to El-Nahas and El-Ashmawy (2004), the observed decrease in reproductive organ weights caused by metronidazole can be attributed to a reduction in testosterone levels. According to Kumari and Singh (2013), administration of the drug at a high dosage resulted in notable decrease in the weights of both the testis and epididymis. Several prior studies have reported that metronidazole induces notable modifications in testicular parameters, including testicular weight, testicular index (the ratio of testicular weight to body weight), and seminiferous tubule diameter (Davood *et al.*, 2007; Samah, 2012; Rhayf *et al.*, 2014). According to a study conducted by Grover *et al.* in 2001, it was observed that the administration of metronidazole (at a dosage of 400 mg/kg/day) to rats for a duration of 6 weeks resulted in reductions in testicular weight, testicular and epididymal spermatozoon counts, as well as the occurrence of abnormal spermatozoa morphology accompanied by seminiferous tubule degeneration. Previous

http://doi.org/10.54328/covm.josvas.2023.137

studies have also documented morphological abnormalities in the flagellum and head, as well as a reduction in the quantity of motile spermatozoa, following treatment with metronidazole (el-Nahas and el-Ashmawy, 2004; Mudry *et al.*, 2007).

Vitamin C (Ascorbic Acid) is a water-soluble antioxidant found in plasma and tissues. It functions as a non-enzymatic antioxidant (Chambial *et al.*, 2013). Zhou *et al.* (2021) demonstrated that ascorbic acid exhibits a protective influence on diverse tissues and organs, encompassing the reproductive system. According to Angulo *et al.* (2011), the antioxidant properties of vitamin C enable it to counteract the harmful effects of Reactive Oxidative Species (ROS), thereby mitigating tissue damage. According to Raymond and Costabile (1998), the maintenance of spermatogenesis in animal models has been demonstrated to be effective. Previous studies have reported the positive effect of Vitamin C on both the quantity and quality of spermatozoa (Ekaluo *et al.*, 2013; Olorunshola *et al.*, 2011), as well as its potential role in increasing testosterone levels (Angulo *et al.*, 2011). The available therapeutic interventions for managing and preventing complications associated with metronidazole are currently constrained in their scope. This study was conducted in order to assess the effect of vitamin C on the reproductive parameters of male rabbits experiencing toxicity induced by metronidazole.

MATERIALS AND METHODS

EXPERIMENTAL ANIMAL, DRUGS AND TREATMENT

Twenty (20) apparently healthy, domestic rabbit bucks (*Oryctolagus cuniculus*) that were between 10 months to 2 years old with average body weight of 1.7 ± 0.2 kg were used for the study. Ethical approval was sought from University of Ilorin Research Ethical Review Committee before commencement of the study (No: UREC/FVM/PG20/68VO002). The animals were housed at the rabbit unit of Federal University, Wukari, Taraba State and feed and water were provided *ad libitum*.

The animals were divided into four groups of five rabbit per group as follows: Group I: Untreated control, Group II: Oral administration of Metronidazole (400 mg/kg BW/day) for 30 days, Group III: Administration of Metronidazole (400 mg/kg BW/day) for 30 days and administration of Vitamin C (200mg/kg BW/day) for another 30 days afterward and Group IV: Administration of Vitamin C (200mg/kg BW/day). The human therapeutic dose of metronidazole was used for the study. Vitamin C (Em-Vit-C®) tablet (100 mg) from Emzor Pharmaceuticals Nig Ltd and metronidazole (200 mg) (Metrone® 200) tablets from Fidson HealthCare Plc. They were first made into a solution by dissolving them in distilled water before use.

BODY AND ORGAN WEIGHT DETERMINATION

The animals were weighed at the end of the experiment using a digital weighing balance (Amazecare TS200, India). Three bucks from each group were humane slaughtered after semen collection and analyses, during which the testes were meticulously removed using a scalpel blade. The weights of both the testis and epididymis were ascertained by separating the epididymis from the testis and weighing them individually using a digital weighing balance (Amazecare TS200, India).

SPERM CHARACTERISTICS DETERMINATION

Semen sample was collected from the bucks through the use of an artificial vagina (AV). The bucks were trained to ejaculate into an artificial vagina (AV). Condoms, sample bottle and syringes were used to improvise an AV. The AV was held beneath the teaser doe with its open end pointing caudally after being pre-heated in a hot water bath. The AV was positioned further caudally as the buck started to mount to enable the rabbit's penis to penetrate the AV; ejaculation happened rapidly after the penis penetrate (Naughton *et al.*, 2003). Warm water was used to maintain the temperature of the ejaculates at 37 °C. The evaluated sperm characteristics included sperm motility, spermatozoa concentration, percentage live-dead ratio and sperm morphology.

Sperm motility was determined by diluting little amount of semen on a pre-warmed glass slide with sodium citrate buffer and examined under the microscope for forward progressive motility and graded as percentage progressive motility, non-progressive motility and no motility. Spermatozoa concentration was determined through the use of Neubauer haemocytometer as described by Christensen *et al.* (2005). Percentage live-dead ratio (%) was determined by mixing one drop of semen with two to three drops of eosin-nigrosin stain on a warm slide as described by Wells and Awa (1970). From the mixture of semen and stain, a thin smear was made and allowed to air dry. The percentage of live and dead sperm cells was determined by counting the live and dead sperm cells individually. Sperm morphology was determined by adding two drops of warmed Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and air-dried; the stained slide was examined under the microscope at × 400 magnification (Wyrobek, 1979). Five fields of the microscope were randomly selected and the types and number of abnormal spermatozoa were evaluated from the total number of spermatozoa in the five fields; the number of normal and abnormal spermatozoa were expressed as a percentage of the total number of spermatozoa.

DATA ANALYSIS

The obtained data were presented as Mean ± SEM using column statistics and were subjected to statistical analysis

using one-way analysis of variance (ANOVA), followed by *Tukey post-hoc* multiple comparison test. The data analysis was performed using Graphpad Prism version 5.03 for Windows, developed by Graphpad Software in San Diego, California, USA. Values of $P \leq 0.05$ were considered significant.

RESULTS

EFFECTS OF VITAMIN C ON BODY WEIGHT OF RABBIT BUCKS EXPOSED TO METRONIDAZOLE TOXICITY

In Table I, the mean (± SEM) values of body weight of rabbit bucks exposed to metronidazole and vitamin C before commencement of experiment and after the experiment are presented. The mean body weight did not differ significantly ($p > 0.05$) among the groups before and after the experiment. The body weight for the control, MTZ, MTZ + Vit C and Vit C groups were 1.72, 1.62, 1.66 and 1.69 kg respectively after the experiment.

EFFECTS OF VITAMIN C ON ORGANS WEIGHT OF RABBIT BUCKS EXPOSED TO METRONIDAZOLE TOXICITY

In table II, the testicular weight and epididymal weight of rabbit bucks in different treatment groups are presented. In the right testes, the mean weight of the control group (1.60g) was significantly ($p \leq 0.05$) higher when compare to the MTZ (1.23g) and MTZ + Vit C (1.52g) groups. The mean weight in the MTZ + Vit C group was higher when compare to the weight in the MTZ. This indicates significant ($p \leq 0.05$) improvement following Vit C administration in the MTZ + Vit C group. In the left testes, the mean weight in the MTZ group (1.24g) was significantly ($p \leq 0.05$) lower in comparison to the control (1.62g) and Vit C (1.67) groups. The mean weigh in the MTZ +Vit C (1.56g) group was higher than the mean weight in the MTZ group indicating an improvement. The mean weight recorded for the right epididymis was lower in the MTZ (0.16g) group when compared to 0.24g for the control. The MTZ + Vit C group recorded a mean of 0.20g which is higher than 0.16g in the MTZ group. The mean weight of left epididymis in the control group (0.25g) was significantly ($p \leq 0.05$) higher than that of the MTZ (0.15g) and MTZ + Vit C groups. MTZ + Vit C recorded a higher mean of 0.20 in comparison to the MTZ group signifying an improvement.

EFFECTS OF VITAMIN C ON GONADAL AND EPIDIDYMAL SPERM RESERVE OF RABBIT BUCKS EXPOSED TO METRONIDAZOLE TOXICITY

Table III showed gonadal sperm reserves of rabbit bucks in control, MTZ, MTZ + Vit C and Vit C groups. The gonadal sperm for the control group was 21.50 $x10^6$/g. There was significant ($P<0.05$) decrease in the right testicular sperm reserve of the MTZ (5.75 $x10^6$/g) when compare to the Control (21.50 $x10^6$/g). A significantly higher figure was recorded in the MTZ + Vit C (10.75 $x10^6$/g) when compared to the MTZ. There was significant ($P<0.05$) decrease in the left testicular sperm reserve of the MTZ (6.00 $x10^6$/g) when compare to the Control (19.50 $x10^6$/g). MTZ + Vit C was higher than the MTZ group.

The sperm reserves in the right epididymis was significantly ($P<0.05$) higher in control (21.00 $x10^6$/g) when compared to MTZ group (7.00 $x10^6$/g). The value for MTZ + Vit C (13.50 $x10^6$/g) was not significant when compared to the MTZ but was observed to be higher. The left epididymis also recorded a significantly ($P<0.05$) lower value of 6.25 $x10^6$/g in the MTZ group when compared to the control (21.25 $x10^6$/g) and 13.50 $x10^6$/g in the MTZ + Vit C when compared to the MTZ group (Table III).

Table I: Effects of Vitamin C on body weight of rabbit bucks exposed to metronidazole toxicity

	Control	MTZ	MTZ + Vit C	Vit C
Before Experiment	1.69 ± 0.04	1.61 ± 0.03	1.65 ± 0.06	1.67 ± 0.04
After Experiment	1.72 ± 0.04	1.62 ± 0.04	1.66 ± 0.05	1.69 ± 0.09

($p > 0.05$)

Table II: Effects of Vitamin C on organs weight of rabbit bucks exposed to metronidazole toxicity

Organs	Control	MTZ	MTZ + Vit C	Vit C
Right Testes	1.60 ± 0.02	1.23 ± 0.07[a]	1.52 ± 0.04[a]	1.67 ± 0.04[b]
Left Testes	1.62 ± 0.03	1.24 ± 0.06[a]	1.56 ± 0.07	1.67 ± 0.02[b]
Right Epididymis	0.24 ± 0.03	0.16 ± 0.02	0.20 ± 0.01	0.26 ± 0.02
Left Epididymis	0.25 ± 0.03	0.15 ± 0.01[a]	0.20 ± 0.01	0.28 ± 0.01[b]

[a] Significant difference compared to control ($P<0.05$).
[b] Significant difference compared to MTZ ($P<0.05$).

EFFECT OF VITAMIN C ON SPERM PARAMETERS OF RABBIT BUCKS EXPOSED TO METRONIDAZOLE

The percentage sperm motility in MTZ group (32.50%) reduced significantly ($p<0.05$) when compared to that of Control (75.83%). There was significant ($p<0.05$) increase in the percentage sperm motility observed in MTZ + Vit C group (61.33%) when compared to MTZ group. The percentage sperm in Vit C group was 77.33% (Table 1). Sperm count was significantly ($p<0.05$) lower in MTZ group (58.67 $x10^6$/ml) as compared to the Control (141.67 $x10^6$/ml), Vit C (77.33 $x10^6$/ml) and MTZ + Vit C (123.33 $x10^6$/ml) groups. Also a significant ($p<0.05$) increase was recorded in MTZ + Vit C group when compared to the MTZ group (Table IV). There percentage sperm livability in the MTZ group (41.83%) was significantly ($p<0.05$) lower when compared to the control group with 91.50%. The percentage in MTZ + Vit C group (64.17%) was significantly (($p<0.05$) higher when compared to MTZ. Vitamin C improved sperm livability in rabbit bucks exposed to metronidazole. The percentage livability of 92.83 was recorded in Vit C group. The total percentage abnormalities of spermatozoa was significantly ($p<0.05$) higher in the MTZ group (76.50%) when compared to the control (8.33%) and Vit C (7.33%) groups. In the group administered MTZ + Vit C there was significantly lower (27.17%) sperm abnormalities when compared to the group administered MTZ.

Table III: Effect of vitamin C on sperm parameters of rabbit bucks exposed to Metronidazole

Parameters	Control	MTZ	MTZ + Vit C	Vit C
Sperm Motility (%)	75.83 ± 2.20	32.50 ± 11.85[a]	61.33 ± 3.18[b]	77.33 ± 1.76[b]
Sperm Count ($\times10^6$/ml)	141.67 ± 12.42	58.67 ± 6.64[a]	123.33 ± 10.17[b]	156.00 ± 10.21[b]
Sperm Livability (%)	91.50 ± 1.89	41.83 ± 5.92[a]	64.17 ± 3.42[ab]	92.83 ± 2.73[b]
Sperm Abnormalities (%)	8.33 ± 0.17	76.50 ± 0.00[a]	27.17 ± 3.94[ab]	7.33 ± 1.01[b]

[a] Significant difference compared to control ($P<0.05$).
[b] Significant difference compared to MTZ ($P<0.05$).

Table IV: Effects of Vitamin C on gonadal and epididymal sperm reserve of rabbit bucks exposed to metronidazole toxicity

Parameters	Control	MTZ	MTZ + Vit C	Vit C
Testes ($\times10^b$/g)				
Right	21.50 ± 0.50	5.75 ± 0.25[a]	10.75 ± 1.25[a]	16.75 ± 2.25
Left	19.50 ± 1.00	6.00 ± 1.50[a]	12.00 ± 2.00	22.00 ± 0.00[b]
Epididymis ($\times10^6$/g)				
Right	21.00 ± 0.00	7.00 ± 1.00[a]	13.50 ± 0.50	22.00 ± 3.00[b]
Left	21.25 ± 0.25	6.25 ± 0.75[a]	14.75 ± 1.75[ab]	23.5 ± 0.00

[a] Significant difference compared to control ($P<0.05$).
[b] Significant difference compared to MTZ ($P<0.05$)

DISCUSSION

The study found out that there was no significant difference in the body weight of rabbit bucks administered metronidazole and vitamin C before and after the experiment (Table I). This could be due to the absence of androgenic properties in the metronidazole since it has been reported that androgen possess anabolic activities (Johnson & Everitt, 1998). It could also be due to absence of anorectic and lipolytic properties in this drug (Carvajal *et al.*, 2009). This finding is similar to that of Oyedeji *et al.* (2015) and Kumari & Singh (2015) who found in their separate researches no significant changes in the body weight of rabbit bucks exposed to metronidazole.

The study also found decrease in the testicular and epididymal weight in rabbit bucks administered metronidazole (Table II). This may be related to oxidative damage to the testis and subsequent impairment of optimal testicular activity (Akorede *et al.*, 2020). The decline in the testicular and epididymal weights was restored following administration of Vitamin C. The weight of the testis is largely dependent on the mass of the differentiated spermatogenic cells; the reduction in the weight of the testis may be due to decreased number of germ cells, inhibition of spermatogenesis and steroidogenic enzyme activity (Chapin *et al.*, 1997; Takahashi & Oishi, 2001, Farombi *et al.*, 2008). Simmons *et al.* (2002) indicated that reduction in weight or a change in either absolute or relative organ weight after administration of a chemical is an indication of the toxic effect of such chemical or agent. Vitamin C mitigated the effect of metronidazole on the testicular and epididymal weight affected by the drug. This is similar to the finding of Farombi *et al.* (2008) who observed improvement in the testicular and epididymal weight of rats affected by tetracycline-induced reproductive toxicity. Similarly, Benabbou *et al.* (2017) also found out that vitamin C was helpful in restoring decreased testicular weight in wistar rats.

The result on sperm motility showed a significant ($P \leq 0.05$) decrease in the percentage sperm motility in the metronidazole group which

improved in the metronidazole and vitamin C group (Table IV). This is consistent with findings of Oyedeji *et al.* (2015) who found a significant decrease in the sperm motility in wistar rat administered 400 mg of metronidazole. Kumari *et al.* (2013) found a significant decrease in sperm motility of male mice following metronidazole administration.

Sadeghzadeh *et al.* (2019) found a significant increase in the percentage of sperm motility in mice treated with Vitamin C after exposure to dexamethasone. Akorede *et al.* (2020) also found a significant increase in sperm motility in wistar rat exposed to carbamazepine. Metronidazole caused a decrease in sperm motility which improves with administration of Vit C.

The study found a significant decrease ($P \leq 0.05$) in sperm count in the metronidazole group in comparison to the control, Vit C and MTZ + Vit C groups. This finding is in consonance with the findings of Kumari *et al.*, (2013), Singh *et al.*, (2013) and Oyedeji *et al.*, (2015) who all found significant decrease in sperm count after administration of metronidazole. Administration of vitamin C to rabbit bucks after one month of metronidazole administration caused an improvement in the sperm count. This is in agreement with the Akorede *et al.,* (2020) who found an improvement in sperm count of wistar rats administered carbamazepine and treated with vitamin C. Shabanian *et al.* (2017) also found vitamin C useful in ameliorating reproductive toxicity caused by cyclophosphamide exposure in rats.

The percentage sperm livability is significantly low for the MTZ group as compared to the control, Vit C and MTZ + Vit C (Table III). This result showed that metronidazole affects the process of spermatogenesis in the sertoli cells of the testis. This result is consistent with the findings of Oyeyemi *et al.* (2014) who found percentage increase in sperm livability in rat exposed to nicotine and vitamin C in comparison to those exposed to nicotine alone. Shabanian *et al.* (2017) had similar finding with vitamin C and cyclophosphamide.

The result of percentage sperm abnormalities showed that the percentage abnormal sperm cells were higher in the MTZ group when compared to the control, Vit C and MTZ + Vit C groups (Table III). This result was corroborated by previous researches such as that of Oyeyemi *et al.,* (2014) who found vitamin C helpful in ameliorating decrease in sperm abnormalities caused by nicotine administration in rats and Akorede *et al.* (2020) who reported a decrease in sperm abnormalities after vitamin C administration in carbamazepine-induced reproductive toxicity. Also, it has been reported that dietary supplement of Vitamin C improved sperm quality (Luck *et al.,* 1995). The beneficial effects of vitamin C seen in this study may result from its antioxidant activity in mopping metronidazole induced free radicals (Dietrich *et al.,* 2003).

The mean sperm reserve in the right testes is significantly lower in the MTZ and MTZ + Vit C groups when compared to the control and vitamin C groups (Table IV). The sperm reserves in the left, right testes and testes were significantly decreased or adversely affected by metronidazole in male rabbits. Kumari and Singh (2015) reported decreased sperm reserve in rabbit bucks administered metronidazole. Farombi *et al.* (2008) found an improvement in sperm reserve in male rats after vitamin C administration following exposure to tetracycline.

The reason for the improvement in the testicular sperm reserve may be because of the antioxidant properties of vitamin C. The result of the study also showed significant decreased sperm reserve in the epididymis. Both the right and left testes had a significant decrease in sperm reserve in the metronidazole group compared to the other groups. Metronidazole + vitamin C group improved after administration of vitamin C. This result showed that metronidazole caused the decrease in the epididymal sperm reserve of the rabbit bucks. Previous researches has found decrease in epididymal sperm reserve following prolong administration of metronidazole (Davood *et al.,* 2007, Rhayf *et al.,* 2014 and Oyedeji *et al.,* 2015). Similarly, other researchers have reported improvement in the mean value of epididymal sperm reserve following vitamin C administration (Farombi *et al.,* 2008, Oyeyemi *et al.,* 2014 and Shabanian *et al.,* 2017).

CONCLUSION

The results of the present study indicate that metronidazole treatment in rabbit bucks induces testicular injury as is evident from the changes in the testicular and epididymal weights sperm characteristics (sperm motility, sperm count, sperm viability and sperm morphology) and testicular and epididymal sperm reserve. Vitamin C as an antioxidant was observed to have ameliorated all the adverse effects of metronidazole treatment on the testis structure and therefore its application in therapeutic regimens including metronidazole administration is recommended.

ACKNOWLEDGEMENT

The authors appreciate the HOD Animal Production and Health, Federal Unversity, Wukari Dr Kayode Onaleye for granting us the approval to use the rabbit unit in the Research Farm for the experiment.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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