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Protective effects of vitamin C on hormonal level and testicular histopathology of rabbit bucks with metronidazole-induced toxicity

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ABSTRACT

Objective: This study evaluates the protective effects of Vitamin C on hormonal level and testicular histopathology in rabbit bucks with metronidazole-induced toxicity. Methods: Twenty adult rabbit bucks which were weighed and divided into four groups with five in each group were used for the study. Group I is the control, group II was given metronidazole 400 mg/kg/day for 30 days, group III was co-administered 400 mg/kg/day of metronidazole and 200 mg/kg/day of Vitamin C for 30 days and group IV was given 200 mg/kg/day Vitamin C for 30 days. At the end of the experiment, the rabbit bucks were weighed, and blood samples were collected from the marginal ear vein into a plain bottle and serum extracted through centrifugation for hormonal assay. FSH, LH and Testosterone assay were carried out using Enzyme Linked Immunosorbent Assay (ELISA) kits according to manufacturers' instruction. One testis from each rabbit was removed for testicular histology. Results: The study found out that there was no significant difference in the body weights of the rabbit bucks before and after the experiment, metronidazole significantly ($p < 0.05$) affects hormonal concentration in the bucks and there was significant improvement following vitamin C administration. The study also found out that metronidazole caused testicular degeneration which was reversed by Vitamin C administration. Conclusion: Vitamin C has protective effect against metronidazole-induced toxicity and its use in therapeutic application in prolong use of metronidazole is recommended.

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Introduction

Metronidazole (MTZ) is a pharmaceutical agent with antiprotozoal and antibacterial properties, which finds widespread application in the field of veterinary medicine. The antibiotic has been utilised in human and veterinary medicine for the therapeutic management of trichomoniasis, giardiasis, amebiasis, and anaerobic bacterial infections (Bergan, 1985). However, the prolonged utilisation of the drug has been documented to induce adverse effects on reproductive functions in rabbits (Foote, 2002). According to Castellini (2008), reproductive toxicity has been associated with many negative impacts on the reproductive system, such as diminished fertility rates, compromised semen quality, reduced hormone concentration, and other detrimental effects. According to a study conducted by Rhayf et al. (2014), it has been documented that metronidazole can induce reproductive toxicity in male rabbits. According to Rhayf et al. (2014), the administration of metronidazole resulted in a notable reduction in the weight of the testes, epididymis, and seminal vesicles. Additionally, it led to a decline in sperm count, motility, viability and reduced testosterone concentration. In a study conducted by Oyedeji et al. (2015), notable alterations were observed in the viability of sperm and a slight degradation of the germinal epithelia of the testes in Wistar rats.

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According to Roy et al. (2014), Vitamin C (Vit C) exhibits strong antioxidant properties and has demonstrated a protective influence against genotoxicity caused by metronidazole in mice. Vitamin C has been found to have a protective effect on spermatogenesis and is essential for maintaining semen integrity and fertility in both humans and animals. Additionally, it has been observed to elevate blood testosterone levels (Fernandes et al., 2011; Sonmez et al., 2005). According to a study conducted by Benabbou et al. (2017), the administration of Vitamin C has been found to have beneficial effects in mitigating the adverse effects of different drugs and substances on the reproductive system. Furthermore, it has been demonstrated in a study conducted by Sadeghzadeh et al. (2018) that vitamin C has the ability to mitigate the detrimental impacts of dexamethasone on sperm motility, testosterone levels, and spermatogenesis indexes in mice. According to a study conducted by Akorede et al. (2020), the administration of vitamin C had a protective effect against the modification of many parameters associated with oxidative alterations, sex hormones, sperm characteristics, relative pituitary and testicular weight, as well as histological changes.

Previous studies have suggested that vitamin C supplementation is beneficial in reducing the toxic effects of various drugs and chemicals on the reproductive system (Sadeghzadeh et al., 2018, Akorede et al., 2020, Hajjar et al., 2020). However, the potential of vitamin C in reducing the reproductive toxicity induced by metronidazole has not been thoroughly investigated, hence, the need for this study to evaluate the protective effect of Vitamin C on the hormonal level and testicular histopathology of male rabbits exposed to metronidazole.

Materials and Methods

Ethical approval

Ethical approval was sought from University of Ilorin Research Ethics Committee with approval No.: UREC/ FVM/PG20/68VO002 before commencement of the research and ethical guidelines for the use of animal was strictly followed.

Experimental animals and treatment

A total of twenty mature and apparently healthy, domestic rabbit bucks were used for this study. The rabbits had an average weight ranging from 1.6 to 1.8 kg. They were housed at the rabbit unit of the Faculty of Agriculture Research and Demonstration Farm of Federal University Wukari. The rabbit bucks were given unrestricted access to both feed and water. Prior to the start of the experiment, they were allowed to acclimatized for two weeks. The twenty rabbit bucks were divided into four groups with five bucks in each group. Group I was control, group II rabbit bucks were given oral administration of metronidazole (400 mg/kg BW/day) for 30 days, group III were co-administered 400 mg/kg BW/day of metronidazole and 200mg/kg BW/day oral dose of vitamin C for 30 days and group IV were given 200mg/kg BW/day dose of vitamin C for 30 days. Vitamin C tablet (Em-Vit-C® from Emzor Pharmaceuticals Nig Ltd) and metronidazole tablets (Metrone® 200 from Fidson Healthcare Plc) were purchased from New World Pharmacy in Wukari, Taraba State, Nigeria for the experiment. The drugs were first made into a solution by dissolving them in distilled water using 1ml of distilled water for each tablet before use. At the end of the treatment period, the rabbit bucks were weighed, and 3 mL of blood was obtained using a 5 mL syringe and a 23 G needle from the marginal ear vein of each rabbit. The blood was collected into a plain sample container. The blood samples were centrifuged at 3000 rpm for 5 minutes in order to obtain sera samples, which were subsequently used for hormonal level analysis. The rabbits were subjected to a humane slaughter, following which the testes were extracted using a scalpel blade and subsequently preserved in a solution of 10% formalin for testicular histology.

Evaluation of sex hormones concentration

Serum FSH, LH and testosterone were measured using Enzyme Linked Immunosorbent Assay (ELISA) kits obtained from Elabscience® (Texas USA) according to the manufacturer's instructions provided with the kits.

Evaluation of histological changes

The histological examinations of the testes of the rabbits were conducted using the methodology of Shyr et al. (2002). Each subject's testicular tissue was routinely prepared for paraffin embedding after being preserved in 10% formalin for 48 hours. Hematoxylin and eosin (H and E) staining was applied to the embedded tissues after they were serially sectioned using a Rotary Microtome at a 4µm thickness. The tissues were then treated in an alcohol-xylene series. The prepared slides were examined under the microscope at a magnification of x 400.

Statistical analysis

GraphPad Prism Version 5.03 for Windows developed by Graphpad Software in San Diego, California, USA was used to analyze the data. The concentration of sex hormones data was presented as mean ± SEM. They were tested using Tukey's post-hoc multiple comparison test after undergoing a one-way analysis of variance (ANOVA). Every value at $p < 0.05$ was deemed significant.

Results

Effects of Vitamin C on body weight of rabbit bucks exposed to metronidazole toxicity: Table 1 presents the mean (± SEM) values of body weight of rabbit bucks before commencement of experiment and after the experiment. 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 1.69kg respectively after the experiment.

E**ffects on sex hormonal concentration**

Effect of treatment on follicle stimulating hormone (FSH) concentration: The result in Table 2 shows the mean follicle stimulating hormone (FSH) concentrations in the different treatment groups. FSH concentration was significantly ($p < 0.05$) higher in the control group when compare to MTZ and MTZ + Vit C groups. Also, FSH concentration was significantly lower in MTZ group when compare with Control, MTZ + Vit C, and Vit C groups. FSH concentration was significantly ($p < 0.05$) lower in MTZ + Vit C when compare with Vit C group. The result indicates that MTZ caused a significant ($p < 0.05$) decrease in the FSH concentration as compared to the control group. There was significant improvement in the MTZ + Vit C group.

Effect of treatment on luteinizing hormone (LH) concentration: Table 2 also shows the mean Luteinizing Hormone (LH) concentrations in the different treatment groups. The LH concentration was significantly ($p < 0.05$) higher in the control group when compare to MTZ and MTZ + Vit C groups. The mean LH concentration in the MTZ group was significantly ($p < 0.05$) lower when compare to that of Control, MTZ + Vit C, and Vit C groups. Mean LH concentration in MTZ + Vit C was significantly ($p < 0.05$) lower when compare with Vit C group. The result indicates that MTZ caused a significant ($p < 0.05$) decrease in the LH concentration as compared to the control group. There was significant improvement in the MTZ + Vit C group.

Effect of treatment on testosterone concentration: Table 2 also shows the mean testosterone concentrations in the different treatment groups. The mean testosterone was significantly ($p < 0.05$) higher in the control group when compare to the MTZ and MTZ + Vit C groups. The mean testosterone concentration in the MTZ group was significantly ($p < 0.05$) lower when compare to that of control, MTZ + Vit C, and Vit C groups. Testosterone concentration in MTZ + Vit C group was significantly ($p < 0.05$) lower when compare with Vit C group. The result indicates that MTZ caused a significant ($p < 0.05$) decrease in the testosterone concentration as compared to the control group. There was significant improvement in the MTZ + Vit C group.

Effects of treatment on testicular histology: From the result in Figure 1, the photomicrograph showed a structure of the testis tissue, and the germinal epithelium was observed to be intact and normal in the control group (Plate A). In the MTZ group (Plate B), there were irregular and degenerated seminiferous tubules and a less compact arrangement of spermatogenic cells was observed in the structure of the testis. This could be due to the effect of the metronidazole on the testes. Meanwhile, the MTZ + Vit C group (Plate C) presented restoration of the germinal cells of the seminiferous tubules. Moreover, the Vit C group (Plate D) showed regular seminiferous tubules similar to the control group. Vitamin C was seen to have improved the histological changes observed in the MTZ group

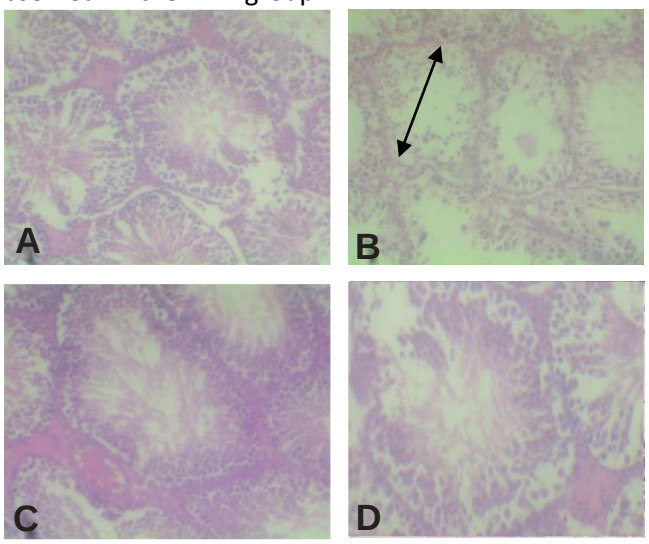

Figure 1: Photomicrograph of the testis of rabbit bucks exposed to Metronidazole and Vitamin C
A: Control, B: MTZ, C: MTZ + Vit C, D: Vit C (H&E x400). A: No visible histological changes. B: Degenerative changes in the seminiferous tubules (Black arrow), less populated with spermatogenic cells, C: Restoration of seminiferous tubules histoarchitecture, D: No visible histological changes.

Table 1. 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 2. Mean ± SEM of serum FSH, LH and testosterone concentrations in different treatment groups

	Control	MTZ	MTZ + Vit C	Vit C
FSH (mIU/mL)	0.836 ± 0.01[a]	0.296 ± 0.04[b]	0.689 ± 0.03[c]	0.852 ± 0.03
LH (mIU/mL)	0.833 ± 0.02[a]	0.331 ± 0.03[b]	0.675 ± 0.03[c]	0.879 ± 0.03
Testosterone (ng/mL)	2.859 ± 0.02[a]	1.674 ± 0.05[b]	2.470 ± 0.05[c]	2.918 ± 0.04

MTZ = Metronidazole. MTZ + Vit C = Metronidazole + Vitamin C. Vit C = Vitamin C. a = ($p < 0.05$) higher when compare with MTZ and MTZ + Vit C groups. b = ($p < 0.05$) lower when compare with Control, MTZ + Vit C and Vit C groups. c = ($p < 0.05$) lower when compare with Vit C group.

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.

In comparison to the Control, MTZ + Vit C and Vit C treated groups, the FSH and LH concentrations were significantly ($p < 0.05$) lower in the MTZ treated group. This finding is in line with the findings of Davood et al. (2007), who observed a substantial decrease in rats treated with 400 mg/kg of metronidazole as compared to the non-treated control group. Grover et al. (2001) similarly observed a comparable decrease in FSH and LH concentrations after metronidazole treatment. The inhibition of gonadotropic releasing hormone (GnRH) secreted by the hypothalamus by metronidazole may be the cause of the decreased serum concentrations of FSH and LH in the metronidazole-treated group. GnRH aids in releasing gonadotropins (FSH and LH) from the anterior pituitary.

The development of accessory reproductive organs and the process of spermatogenesis are androgen dependent. The quantity and functional state of mature Leydig cells will decline as androgen production declines (Osuntokun et al., 2017). In males, FSH controls seminiferous tubules. Because it maintains Sertoli cell activity, which in turn maintains many aspects of sperm cell maturation, follicle-stimulating hormone (FSH) is also critical to sperm production (Egba et al., 2014). Diminished gonadal function (hypogonadism) may be the outcome of decreased LH or FSH production. Males with the disorder usually show up as lacking a normal number of sperm Egba et al., 2014). Spermatogenesis is directly stimulated by FSH when it binds to receptors in sertoli cells (O'Donnel et al., 1994). According to O'Donnel et al. (1994); Singh et al. (1995), LH stimulates Leydig cells to secrete more testosterone. This testosterone may then act on the Sertoli and peritubular cells of the seminiferous tubules, indirectly stimulating spermatogenesis.

Serum FSH and LH concentrations were found to have significantly improved with supplementation with vitamin C. Sadeghzadeh et al. (2019) have reported that vitamin C can enhance the levels of FSH and LH after dexamethasone administration-induced oxidative damage. By reducing the damage caused by oxidative stress, vitamin C can increase the concentration of glutathione (GSH) and the activity of testicular antioxidant enzymes like catalase and superoxide dismutase, which are vital for sperm survival (Olorunshola et al., 2011, Ekaluo et al., 2013).

This study also showed a decrease in the testosterone serum concentration in the MTZ treated group in comparison to the Control, and MTZ + Vit C and Vit C groups. The results of earlier research (Davood et al., 2007, Samah, 2012, Oyedeji et al., 2015, and Kumari & Singh 2015) are consistent with the finding. Testosterone levels were significantly reduced by metronidazole. This could mean that the drug prevents the mechanism that interferes with the Leydig cells' ability to synthesise testosterone. The inhibitory effect of metronidazole on pituitary gonadotropin (FSH and LH) production may be the cause of the decreased serum testosterone levels in the metronidazole group. Similar to this, direct damage to Leydig cells—possibly as a result of oxidative injury—may account for the decrease in testosterone concentration observed in the metronidazole-treated group (Oliva & Miraglia, 2009). Additionally, it's possible that metronidazole causes a decrease in testosterone concentration because it causes the liver's aromatase enzyme to be produced. The enzyme which converts testosterone to estradiol thereby reducing testostosterone concentration (Vijay et al., 2009).

The testosterone concentration improvement seen in the MTZ + Vit C group may indicate that vitamin C helps to mitigate testicular changes caused by metronidazole. This is because vitamin C possesses

antioxidant properties that have been shown to protect biological tissues from reactive oxygen species. Because vitamin C affects the hypothalamic-pituitary-testicular axis, which raises blood testosterone levels, it may also be related to the improvement in hormone concentration in the MTZ + Vit C (Ashamu et al., 2010). Vitamin C or E deficiency causes oxidative stress in the testes, which interferes with testosterone production and spermatogenesis (Rekha et al., 2009).

When compared to the control and vitamin C groups, histological analysis of the testes of rabbit bucks treated with metronidazole showed increased interstitial space and degradation in the spermatogenic cells lining the seminiferous tubule. The current study confirms the observations of Samah (2012), who reported that metronidazole altered the rat seminiferous tubules. The metronidazole crosses the blood-testis barrier and reaches the germ cells in the seminiferous tubules may be the source of this cell injury and histological changes (Dixon & Lee, 1977). There is evidence that metronidazole damaged DNA strands, causing cell necrosis and death (Samah, 2012). Saad et al. (2018) observed a similar histological effect on the seminiferous tubules. Additionally, Kumari & Singh (2015) found that after administering metronidazole, laboratory mice had altered testicular histology, impaired spermatogenesis, and had smaller seminiferous tubules. The erosion of germinal epithelia has been reported by Oyedeji et al. (2015), which is consistent with the results of our investigation. Given that spermatogenesis is reportedly triggered by testosterone, which is produced by Leydig cells and acts on Sertoli cells and peritubular cells, the low population of germinal epithelium may be the result of insufficient testosterone (Sharpe, 1987).

Additionally, the study revealed that the histoarchitecture of the testes of rabbit bucks in the MTZ + Vit C group was gradually restored. The antioxidant properties of vitamin C might be the cause of this. This aligns with the research conducted by Sadeghzadeh et al. (2018), which demonstrated that Vitamin C can reverse the negative histological changes, such as apoptosis in Leydig cells, degeneration of seminiferous tubules, and a decrease in spermatocyte and spermatid counts, in the testes of mice exposed to dexamethasone. Compared to the MTZ and MTZ + Vit C treated groups, the seminiferous tubule and germ cells in the Vit C group were significantly normal.

Conclusion

This study investigated the protective effects of vitamin C on hormonal levels and testicular 264 histopathology in rabbit bucks that were subjected to metronidazole-induced toxicity. The study found out that metronidazole caused significant deleterious effects on the hormonal concentration of rabbit bucks exposed to the drug for 30 days. The drug also caused degenerative effects on the histoarchitecture of the testes of rabbit bucks. The research has provided valuable findings regarding the therapeutic advantages of vitamin C in alleviating the adverse effects of metronidazole on the reproductive system of rabbit bucks. Vitamin C was fond to be a useful protective agent against the toxic impacts of metronidazole on the hormonal level and testicular histopathology of male rabbits. The study's findings on the protective properties of vitamin C have significant clinical significance in management of reproductive toxicity caused by prolong administration of metronidazole. The findings of the study could potentially guide further detailed research in clinical settings.

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Conflict of Interest

The authors have no conflict of interest.

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