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DOSE-DEPENDENT EFFECTS OF ORAL ADMINISTRATION OF ORANGE JUICE ON EPIDIDYMAL SPERM RESERVE AND SEMEN QUALITY IN ADULT MALE RABBITS

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ABSTRACT

Citrus sinensis (sweet orange) is rich in vitamins, flavonoids, and bioactive compounds with antioxidant and immunomodulatory properties, which may influence growth performance, haematological parameters, and male reproductive function. This study evaluated the effects of graded doses of *Citrus sinensis* juice on growth performance, haematology, immune response, and epididymal sperm reserve in adult male rabbits. A total of twenty (20) rabbits were assigned to control, low (3 ml), medium (5 ml), and high (10 ml) juice dose groups and treated for six weeks. Body weight, average daily gain, haematological indices, white blood cell counts, and epididymal sperm parameters were assessed. Administration of *Citrus sinensis* juice resulted in a dose-dependent increase in body weight gain and average daily gain, particularly in the medium and high-dose groups, although differences were not statistically significant ($p > 0.05$). Haematological parameters, including packed cell volume and haemoglobin, remained stable, while white blood cell counts increased significantly ($p < 0.05$) in medium and high-dose groups, indicating enhanced immune activity without pathological lesions. Epididymal sperm reserve and morphometric indices exhibited trends toward improvement at higher doses, consistent with the antioxidant-mediated protection of spermatozoa; however, these changes were generally not statistically significant ($p > 0.05$). Some variability in sperm motility and morphometric parameters suggests dose-dependent and individual physiological responses. *Citrus sinensis* juice supplementation may support growth, maintain haematological stability, enhance immune function, and potentially improve male reproductive parameters in rabbits. Observed variability underscores the need for further studies to optimize dosing and clarify mechanisms of action.

Keywords: *Citrus sinensis*, Epididymal sperm reserve, Male fertility, Semen quality, Rabbits

INTRODUCTION

Sweet orange (*Citrus sinensis* L.) is one of the most widely consumed fruits globally, valued for its high content of dietary antioxidants, including vitamin C (ascorbic acid), flavonoids (such as hesperidin, narirutin, and naringin), and carotenoids. These bioactive compounds are present in the fruit's edible pulp and juice and are known to exert significant antioxidant, anti-inflammatory, and metabolic effects that can influence systemic physiology, including male reproductive function (Klimczak et al., 2007; Li et al., 2016; Agarwal et al., 2019).

The current study is grounded in the Antioxidant Protection Theory, which posits that antioxidants, such as those in orange juice, can mitigate oxidative stress by neutralizing reactive oxygen species (ROS) that damage sperm membrane lipids, mitochondria, and DNA. Excessive ROS generation in the male reproductive tract impairs sperm motility, viability, and concentration, ultimately compromising fertility. By enhancing the antioxidant defence system, dietary supplementation with orange juice may improve epididymal function and protect spermatozoa from oxidative injury, thereby increasing sperm reserve and overall semen quality (Agarwal et al., 2019; Cai et al., 2021).

Previous animal studies have shown that supplementation with orange juice or its bioactive constituents leads to improvements in sperm motility, count, morphology, and testicular histology, mainly due to the action of vitamin C and flavonoids (Jimoh, Ayedun, Ayodele, Omoniyi, Oladepo, Lawal, Ademola, & Kolawole, 2020). Additionally, citrus peel extracts and other phytochemicals have been reported to support Leydig cell function, testosterone synthesis, and overall reproductive efficiency in male rabbits (El-Gindy, Sabir, & Zahran, 2023).

Despite these documented benefits, there is limited and sometimes inconsistent evidence on the dose-dependent effects of whole orange juice on specific reproductive endpoints such as epididymal sperm reserve (ESR) in adult male rabbits. The antioxidant content of orange juice can vary by variety, maturity, and processing, and excessive intake may potentially lead to pro-oxidant or cytotoxic effects under certain conditions (Klimczak *et al.*, 2007; Agarwal *et al.*, 2019).

Therefore, the aims of this study are to evaluate the effects of oral administration of orange juice at different doses on epididymal sperm reserve and semen quality in sexually mature male rabbits. The study also examines associated changes in haematological parameters and body weight to ensure overall physiological safety and to clarify the optimal dosing for reproductive enhancement.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Ripe sweet oranges (*Citrus sinensis*) were purchased from Gomboru Market, Maiduguri, Borno State, Nigeria, and were originally sourced from Ngala Local Government Area. The

fruits were thoroughly washed under clean running water to remove dirt and contaminants. The peels were manually removed, and the edible portions were homogenized using a food-grade blender. The homogenate was then filtered through a clean muslin cloth to obtain the juice extract. The extract was stored at 4 °C in a refrigerator until use for experimental administration.

Ethical Approval and Animal Welfare
All experimental procedures were conducted in accordance with standard guidelines for the care and use of laboratory animals and were approved by the Animal Use and Ethics Committee (AUEC), Faculty of Veterinary Medicine, University of Maiduguri.

Experimental Animals, Housing, and Management
A total of twenty (20) sexually mature New Zealand White rabbit bucks (or locally adapted strains), aged between 5–7 months and weighing 1.5–2.5 kg, were used for this study. The animals were obtained and housed in the animal facility of the Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri. The rabbits were allowed to acclimatize for a period of two (2) weeks prior to the commencement of the experiment. During this period, they were maintained under standard laboratory conditions with adequate ventilation, temperature, and light cycles. Feed and clean drinking water were provided *ad libitum*. The animals were randomly divided into four (4) experimental groups (Groups A, B, C, and D), with five (5) rabbits in each group.

Semen and Haematological Evaluation
Following acclimatization, the rabbits were transported to the Theriogenology (Artificial Insemination) Laboratory of the Large Animal Clinic, University of Maiduguri, for semen evaluation. Blood samples were collected from the marginal ear vein using capillary tubes for haematological analysis. Packed Cell Volume (PCV) was determined using a microhematocrit centrifuge and read with a haematocrit reader, while White Blood Cell (WBC) counts were determined using a hemocytometer.

Anaesthesia and Surgical Procedure
The rabbits were sedated with xylazine at a dose of 5 mg/kg body weight and anaesthetized with ketamine at 20 mg/kg body weight. Both agents were administered intramuscularly in a single syringe. Following adequate anaesthesia, bilateral orchiectomy (castration) was performed under aseptic conditions. The testes were surgically excised for further evaluation.

Semen Collection and Evaluation
The cauda epididymis of each excised testis was carefully isolated and punctured using a 21-gauge needle to release epididymal sperm. Semen samples were placed on clean glass slides for immediate microscopic examination of sperm motility. Additional smears were stained using eosin–nigrosine stain to assess sperm viability (live/dead ratio) and morphology. Sperm parameters evaluated included: Sperm motility, Sperm concentration, Progressive motility, Sperm morphology, Sperm viability. All analyses were conducted according to standard laboratory protocols.

Testicular Measurements and Sperm Count
Excised testicular tissues were weighed using a digital weighing balance, and gross abnormalities were recorded. Morphometric parameters, including testicular length and width, were measured using a measuring tape. The cauda epididymis was homogenized, filtered, and diluted appropriately. Sperm concentration was determined using a Neubauer hemocytometer by counting sperm cells in designated grid areas. Results were expressed as $\times 10^6$ sperm cells per cauda epididymis and per gram of tissue.

Data Analysis
All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS (version 2020; IBM Corp., Armonk, NY, USA). Differences among the control and treatment groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test to determine significant differences between group means. The assumptions of normality and homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Body Weight Parameters in Adult Male Rabbits
Graded administration of Citrus sinensis juice resulted in a slight, dose-dependent increase in final body weight, weight gain, and average daily gain in rabbits, with the medium and high doses showing the highest values (Table 1). However, the differences were modest and associated with large variability, suggesting no statistically significant effect ($p > 0.05$) despite a positive biological trend.

Table 1: Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Body Weight Parameters of Experimental Rabbits

Group	Initial Weight (kg)	Final Weight (kg)	Weight Gain (kg)	Average Daily Gain (g/day)
Control	2.09 ± 0.45	2.88 ± 0.19	0.80 ± 0.51	28.43 ± 18.03
Low Dose (3 ml)	2.12 ± 0.58	2.87 ± 0.22	0.80 ± 0.72	28.57 ± 25.75
Medium Dose (5 ml)	2.28 ± 0.48	3.14 ± 0.44	0.86 ± 0.68	30.71 ± 24.17
High Dose (10 ml)	2.19 ± 0.55	3.09 ± 0.13	0.87 ± 0.53	31.12 ± 18.89

Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Haematological Parameters in Adult Male Rabbits
Administration of graded doses of *Citrus sinensis* juice caused mild changes in haematological parameters, with values remaining within normal ranges (Table 2). Packed cell volume (PCV) and haemoglobin showed slight increases, especially at the high dose, indicating a possible improvement in oxygen-carrying capacity. Red blood cell (RBC) counts were lower in treated groups compared to control, while WBC counts increased markedly (Table 2). Overall, the results indicate that orange juice had no adverse effects and may mildly enhance haematological and immune status in rabbits.

Table 2: Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Haematological Parameters of Experimental Rabbits

Group	PCV (%)	Hb g/dL)	RBC 10^6/µL)	WBC (10^3/µL)	Remark
Control	37.0 ± 1.2	12.3 ± 0.4	6.8 ± 0.5	10,400 ± 800	Normal
Low Dose (3 ml)	39.0 ± 1.4	12.3 ± 0.3	3.4 ± 0.2	26,800 ± 1,200	Normal
Medium Dose (5 ml)	37.0 ± 1.3	12.3 ± 0.3	3.9 ± 0.3	65,400 ± 2,000	Normal
High Dose (10 ml)	40.0 ± 1.5	13.3 ± 0.5	3.6 ± 0.3	57,400 ± 1,800	Normal

Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Epididymal Sperm Reserve and Morphometric Parameters in Adult Male Rabbits

Administration of graded doses of Citrus sinensis juice produced variable effects on epididymal sperm reserve and morphometric parameters of experimental rabbits. Across the treated groups, no consistent dose-dependent trend was observed in sperm motility (M), live and dead ratio (L&D), or normal/abnormal morphology (N/W). Similarly, epididymal morphometric parameters such as length and width showed only slight variations between groups. Epididymal sperm reserve ($\times 10^6$) fluctuated across groups without a clear pattern (Table 3), further supporting the absence of a strong dose-related effect.

Table 3: Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Epididymal Sperm Reserve and Morphometric Parameters of Experimental Rabbits

Group	Mass (M, %)	L&D (%)	N/W (g)	Length (cm)	Width (cm)	MVW	Cntn (×10^6)	Average
Control	23	50	50	6.0	11.33	22	111	782
1.1 ml	33	60	60	7.0	11.32	82	1.5	1107
1.25 ml	33	60	60	7.0	11.32	82	1.5	1107
3 ml	16.33	60	30	7.0	1.1	1.1	3.5	3.5
5 ml	31	40	40	36	44	33	13.14	38.11
10 ml	23	50	60	70	0.6	0.53	32	2112

Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Left and Right Testicular Sperm Parameters in Adult Male Rabbits

Figure 1 compares sperm reserves between the left and right testicles across different groups, including control and varying volume treatments (3 ml, 5 ml, 10 ml). The data likely shows how sperm reserves differ between the testicles within each group.

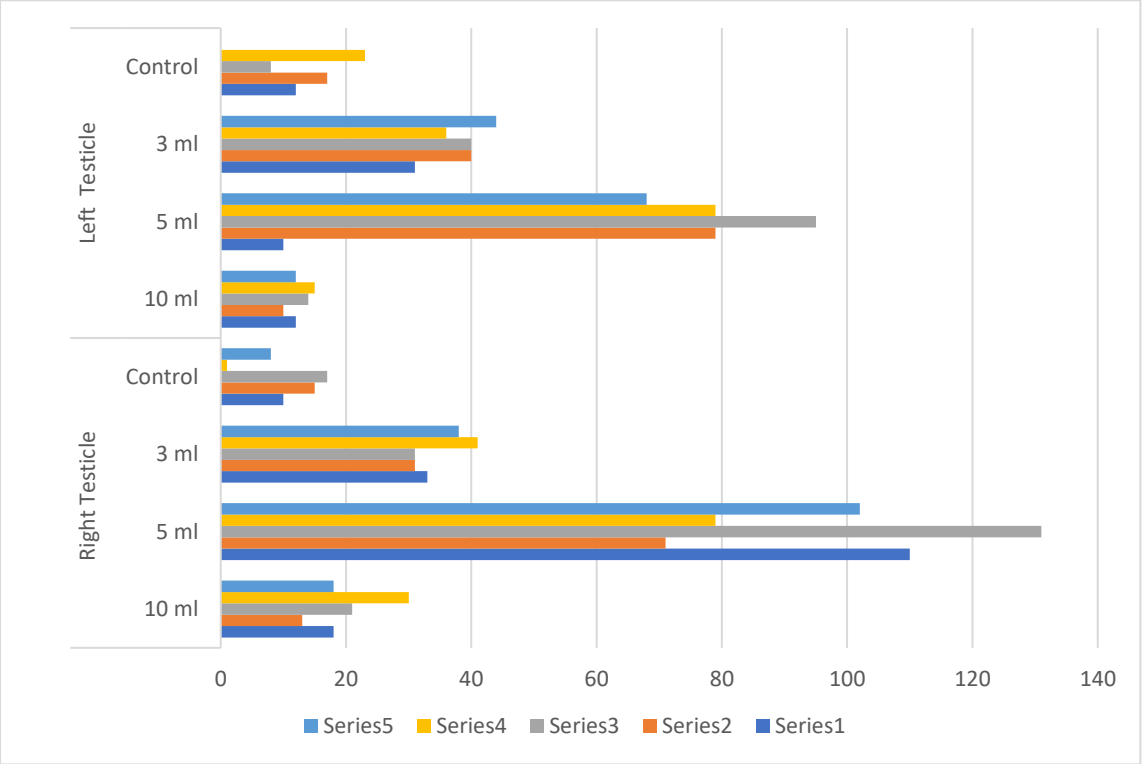

Figure 1: Effect of Graded doses of *Citrus sinensis* (Orange Juice) on Left and Right Testicular Sperm Parameters of Experimental Rabbits

Discussion

Administration of graded doses of *Citrus sinensis* juice resulted in a progressive increase in body weight gain and average daily gain across the treated groups compared with the control. Although the differences were modest and associated with relatively large standard deviations, the consistent upward trend observed, particularly in the medium (5 ml) and high-dose (10 ml) groups, suggests a biologically meaningful improvement in growth performance. This effect may be attributed to the rich nutritional composition of *Citrus sinensis*, which includes simple sugars, vitamins (especially vitamin C), minerals, and flavonoids such as hesperidin and naringin. These phytochemicals have been reported to enhance digestive efficiency, improve nutrient absorption, and modulate metabolic pathways involved in energy utilization and tissue growth (Oluremi et al., 2010; Adewole et al., 2017; Ghasemi et al., 2019). The haematological findings demonstrated that packed cell volume (PCV) and haemoglobin (Hb) concentrations remained relatively stable across all experimental groups, with no statistically significant differences ($p > 0.05$). This indicates that *Citrus sinensis* juice did not exert deleterious effects on erythropoiesis or oxygen-carrying capacity, suggesting that the treatment is largely non-haematotoxin at the administered doses. However, the observed reduction in red blood cell (RBC) counts in treated groups relative to the control, although not statistically significant ($p > 0.05$), may indicate haemodilution or alterations in erythrocyte turnover. This could be associated with the high fluid content of the juice or mild modulation of bone marrow activity (Zhao et al. 2026), suggesting a stimulatory effect on immune function.

In contrast, the elevation in white blood cell (WBC) counts, particularly in the medium and high-dose groups, was statistically significant ($p < 0.05$), suggesting a stimulatory effect on the immune system. Vitamin C is known to accumulate in leukocytes and enhance their functional activities, including chemotaxis, phagocytosis, and microbial killing (Carr and Maggini, 2017). Similarly, citrus flavonoids have been shown to possess immunomodulatory properties, promoting leukocyte proliferation and enhancing host defence mechanisms (Ghasemi et al., 2019). The absence of pathological lesions despite elevated WBC values indicates that the observed leucocytosis is likely physiological rather than indicative of infection or toxicity (Weiss & Wardrop, 2010). The reproductive parameters, including epididymal sperm reserve and morphometric indices, exhibited dose-dependent variations, with indications of improved sperm concentration at higher doses. These changes, however, were not consistently statistically significant ($p > 0.05$), suggesting that orange juice had minimal influence on sperm quality. This trend suggests a potential enhancement of spermatogenesis, which may be mediated by the antioxidant properties of *Citrus sinensis*. Oxidative stress is a major contributor to male infertility, as excessive reactive oxygen species (ROS) can damage sperm DNA, impair membrane integrity, and disrupt spermatogenic processes (Aitken & Baker, 2006). Antioxidants such as vitamin C and flavonoids play a crucial role in scavenging free radicals and protecting testicular tissue from oxidative damage (Agarwal et al., 2014; Aitken and Roman, 2008). Furthermore, vitamin C has been shown to improve sperm count, motility, and overall semen quality by maintaining the structural and functional integrity of spermatozoa (Fraga et al., 1991). The observed improvements in sperm concentration in this study are therefore consistent with these established mechanisms.

However, inconsistencies observed in certain sperm motility parameters and morphometric measurements suggest that the effects of *Citrus sinensis* may not be uniformly beneficial across all reproductive indices. These variations may be attributed to dose-dependent physiological responses, individual variability among experimental animals, or methodological limitations in measurement, indicating that structural integrity of the reproductive organs was largely unaffected by treatment. It is also possible that excessive antioxidant intake may disrupt redox balance, thereby diminishing beneficial effects at higher doses (Helmut et al. 2017). Such findings emphasize the importance of dose optimization in the application of plant-derived substances for reproductive enhancement.

CONCLUSION

Overall, the findings of this study indicate that *Citrus sinensis* juice may exert beneficial effects on growth performance, maintain haematological stability, enhance immune response, and potentially improve male reproductive function in rabbits. These effects are likely mediated through its rich composition of vitamins and bioactive compounds with antioxidant and immunomodulatory properties. Nevertheless, the variability observed in some parameters highlights the need for further studies with larger sample sizes, rigorous statistical validation, and mechanistic investigations to fully elucidate the biological effects and establish optimal dosing strategies.

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