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Evaluation of dietary supplementation of black seed (*Nigella sativa*) on the histological structure of kidney, liver and testis of rabbit bucks

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Abstract

This study investigated the histological response of kidney, liver and testis of rabbit buck to dietary supplementation of Black seed (Nigella sativa). Twenty (20) Chinchilla X New Zealand White, 5-6 weeks old, were balanced for weight and assigned to four (4) treatments T1, T2, T3 and T4 of five rabbits each in a Completely Randomized Design (CRD). The four experimental treatments were supplemented with black seed (Nigella sativa) at 0, 0.5, 1.0 and 1.5% levels, respectively. After twelve (12) weeks of feeding, the bucks were sacrificed and organs (kidney, liver and testes) were extracted for histological evaluation. The results showed that kidney tubular system and the interstitium appeared healthy and normal in the treated groups (T2, T3 and T4) with a mild improvement over the control (T1). The liver hepatocytes were also observed to be normal across the treatments. The seminiferous tubules in testes of rabbit bucks fed varying levels of Black seed had normal and larger well-arranged germ cell population than the control. As Nigella sativa supplementation increased up to 1.5%, the testicular histo-architecture remained normal. This study demonstrated that Nigella sativa seeds, at the supplementation levels in diet, possess the potential to improve and preserve the histo-architecture of rabbit kidney, liver and testis.

Keywords: *Testis, Liver, Kidney, Histology, Black-seed, Rabbit.*

Introduction

Rabbits are widely used in agricultural and biomedical research due to their physiological and anatomical similarities to other mammals (Lossi *et al.*, 2016). Assessing the histological changes in the kidney, liver and testis of rabbit bucks fed supplemented diets can provide valuable information on the safety, efficacy and potential health benefits or otherwise of the dietary intervention (Ezea *et al.*, 2024). Testes are particularly sensitive to oxidative stress and nutritional status, directly impacting male reproductive health. Similarly, the liver and kidneys, as central organs in metabolism and detoxification, are critical indicators of systemic health and the impact of dietary supplements (Alam and Rehman, 2024).

Nigella sativa, commonly referred to as black seed or black cumin, is a flowering plant of the Ranunculaceae family. Its seeds have been traditionally used in various cultures for their wide-range health benefits, including anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective effects

(Manoharan,*et al.*, 2021). The active compounds in black seed, particularly thymoquinone, are reported to exert protective effects against tissue damage caused by oxidative stress and other pathological conditions. These attributes make black seed a promising candidate for improving animal health and productivity (Sadeghi *et al*., 2023)

Histology, the microscopic study of tissues, provides critical insights into the physiological and pathological changes occurring within organs (Pavelka and Roth, 2015). In animal studies, histological evaluations of organs such as the kidney, liver and testis, are vital for understanding the effects of dietary and environmental interventions on their structure and function (Meng *et al*., 2023). Among various natural supplements studied for their therapeutic and protective effects, black seed (*Nigella sativa*), a plant known for its medicinal properties, has gained significant attention (Manoharan, *et al.*, 2021). However, there is dearth of technical information on its use and effects on the histo-architecture of kidney, liver and testis of rabbit buck.

This study therefore, investigated the effects of black seed supplementation on the histological changes in kidney, liver and testis of buck rabbits. By evaluating the structural changes and tissue integrity of these vital organs, this research aimed to elucidate the potential role of black seed in promoting organ health and its implications for reproductive and overall physiological functions in buck rabbits.

Materials and methods

Experimental site

The experiment was carried out at the Rabbit Production and Research unit, Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso. Ogbomoso is situated in a derived savannah zone of southwest of Nigeria and lies on lat. 8° 8' 31.7940" N and long. 4° 14' 42.6696" E. The altitude is between 300m and 600m above the sea level while the mean temperature and annual rainfalls are 27^0C and 1247mm respectively (Ayinla and Odetoye, 2015, Google Earth Map, 2024).

Animals and Management

Twenty (20) weaned rabbit bucks (Chinchilla X New Zealand White, 5-6 weeks old) obtained from a reputable breeding farm in Ogbomoso, Oyo State, Nigeria were used for the experiment. The bucks were individually housed in wooden hutches and subjected to two weeks acclimatization period. They were treated against potential endo- and ecto-parasites and fed diet containing 16% crude protein and average of 2336.12 kcal/kg metabolizable energy. They were balanced for weight and assigned to four treatments of five rabbits each in a completely randomized design. The four treatments were designated as T1, T2, T3 and T4. The rabbit bucks were weighed at the commencement of the experiment and subsequently once per week. They were offered measured quantity of feed with an allowance for them to have left-over (ie *ad libitum).* Cool, clean water was made available throughout the feeding trial which lasted 12 weeks. All routine management practices were well observed. *Nigella sativa*, obtained from a local market in Ibadan, was supplemented in the diets at different levels as follows:

T1 (**Contro**l**):** No *Nigella sativa* supplementation; **T2:** 5g of *Nigella sativa* per kg of diet;

T3: 10g of *Nigella sativa* per kg of diet; **T4:** 15g of *Nigella sativa* per kg of diet i.e 0, 0.5,

1 and 1.5%, respectively. Table 1 shows the gross composition of experimental diets.

Proximate analysis of black seed (Nigella sativa)

The proximate composition of black seed was determined using the conventional procedure of AOAC (2005).

Histological slide preparation

At the end of 12 weeks feeding, three rabbits per treatment were sacrificed their kidney, liver and testis were carefully removed and their samples fixed for histological slide preparation. Samples of kidney and liver were fixed in formol saline while that of testis was fixed in Bouin's fixative. The fixed tissues were dehydrated by dipping in concentrations of alcohol (70%, 80%, 90% and 100%). The tissues were cleared with xylene for about 2 hours, infiltrated with molten paraffin wax at 50^{o}C to 60^{o}C for 2 hours, embedded in molten paraffin wax and labeled appropriately. Thin section of 5µ was cut from the embedded tissues using a microtome (Rotary Microtome RMT-20A). The sectioned tissues were mounted on grease free, clean glass slides, dried at room temperature and stained with Haematoxylin and Eosin (H and E) stain. The tissue section on slides was viewed using light microscope at ×400 magnification. Photomicrograph of the slides were taken using a digital camera, transferred to a computer and labeled appropriately.

Table 1: Gross composition of experimental diets

Feed Ingredients (%)	**T1 Control** **0% *N. sativa***	**T2** **0.5% *N. sativa***	**T3** **1.0% *N. sativa***	**T4** **1.5% *N. sativa***
Maize	32.61	32.61	32.61	32.61
Soybean meal	16.39	15.89	15.39	14.90
N. sativa	0.00	0.50	1.00	1.50
Brewery dry grain	15.00	15.00	15.00	15.00
Rice husk	30.00	30.00	30.00	30.00
Fish meal (72%)	3.00	3.00	3.00	3.00
Oyster shell	2.00	2.00	2.00	2.00
Bone meal	0.25	0.25	0.25	0.25
Vitamin premix*	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15
Methionine	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated Nutrients				
CP (%)	16.20	16.13	16.06	16.00
Metabolizable Energy** (Kcal/kg)	2335.76	2336.00	2336.24	2336.49
CF (%)	13.75	13.75	13.74	13.74

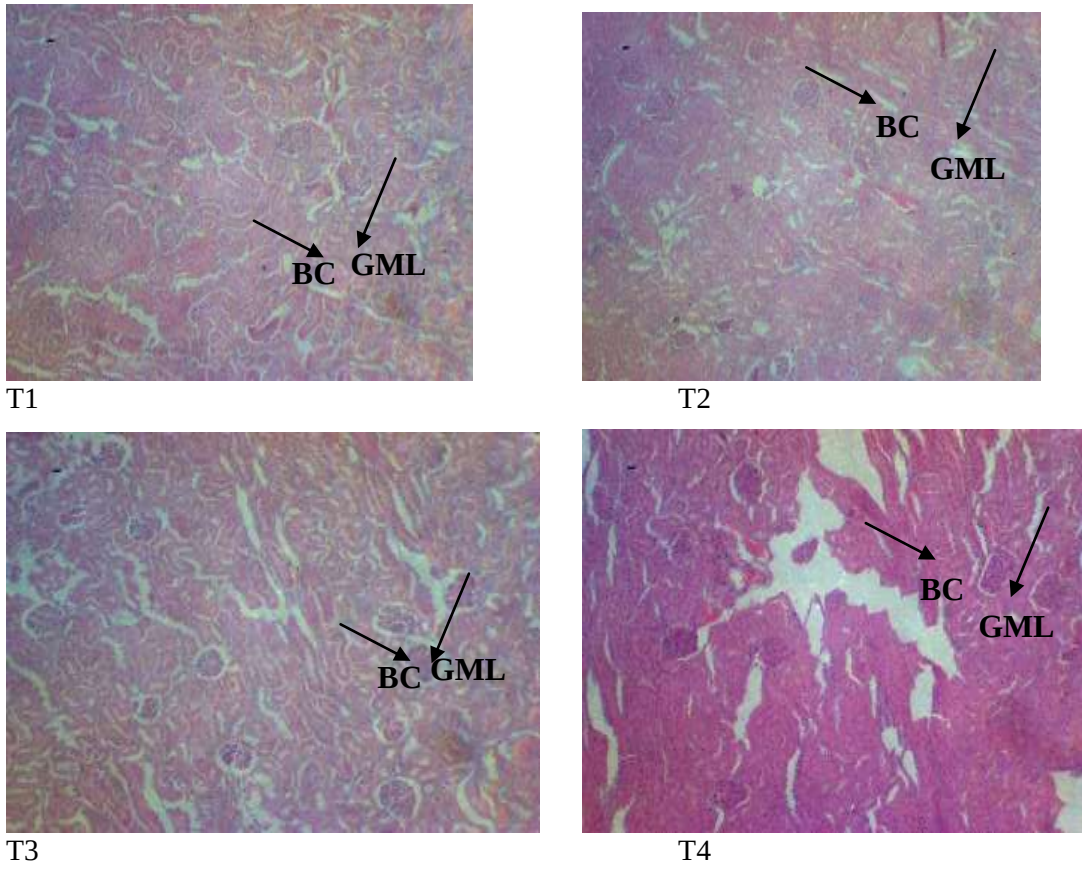
*****Vitamin Premix: Supply per kg diet: 2 000 000 iu vit. A; 400 000 iu D_3; 8.0 g vit. E; 4 g vit. b_1; 1.0 g vit. B_2; 0.6 g vit.; 0.4 mg vit. B_{12}; 24.0 g Niacin; 0.2 g Folic acid; 8.0 g Biotin; 48.0 g Choline; 320.0 g BHT; 16.0 g Manganese; 8.0 g iron; 7.2 g] Zinc; 0.32 copper; 0.25 iodine; 36. 0 mg cobalt; 16.0 mg Selenium. ** Metabolizable Energy calculated using Pauzenga, (1985).

Results

The photomicrographs of the tissues are presented in Plates 1 to 3

Histological structure of the kidney of rabbit buck fed varying levels of Nigella sativa

The histological structures of the kidneys of rabbit bucks fed diets supplemented with *Nigella sativa* are shown in Plate 1. Healthy glomeruli that also appeared many in number were observed in the control treatment (T1). Clearly distinct filtration space and well outlined Bowman's capsules were also observed. The vascular and urinary poles were also distinct. No signs of pathology in the glomeruli, tubular system and interstitium. Proximal and distal tubules were well outlined. In bucks placed on T2 and T3, the kidney histology appeared normal with no signs of pathology. Glomeruli are healthy and numerous. The tubular system and the interstitium appeared healthy and normal, a mild improvement of what was observed in T1. The glomeruli of rabbits fed 1.5% *N. sativa* (T4) appeared healthy, numerous in number and the poles were also distinct. The interstitium was adequate. However, signs of mild tubulorrhexis could be seen.



T1 T2

T3 T4

Plate 1: Photomicrograph of the kidney of rabbit bucks fed diets supplemented with *Nigella sativa*. BC: Bowman capsule; GML: Glomerulus; T1: Control, 0% *N. sativa*; T2: 0.5% *N. sativa*; T3: 1.0% *N. sativa;* T4: 1.5% *N. sativa*.

Histological structure of the liver of rabbit buck fed varying levels of Nigella sativa

The histological structures of the liver of rabbits fed *Nigella sativa* supplemented diets are shown in Plate 2. The structures showed that bucks fed control diet (T1) presented healthy hepatocytes and clear sinusoids. The portal triads and central veins were normal and healthy. Distinct hepatocytes were observed in liver of bucks that were fed 0.5 and 1.0% (T2 and T3, respectively). Their sinusoids were clear, unclogged and well outlined. The portal triads and central vein appeared normal. The histo-architecture was generally normal in these treatment groups. The liver of rabbit bucks in T4 (1.5% *N. sativa*) presented a healthy appearance. However, the sinusoids appeared mildly disrupted. The portal triads, central veins and hepatocytes were normal and healthy in appearance.

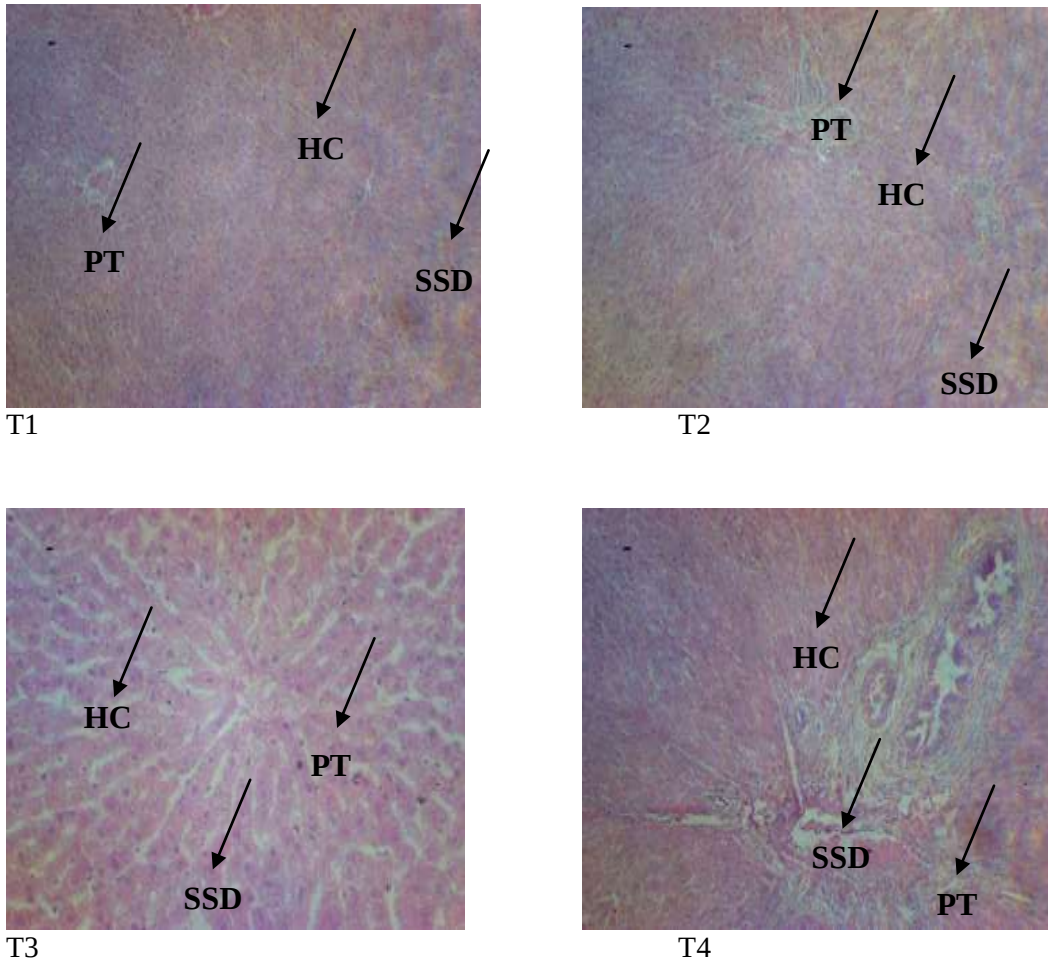
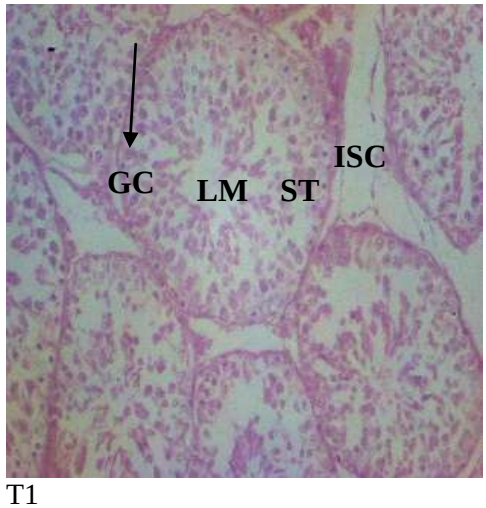


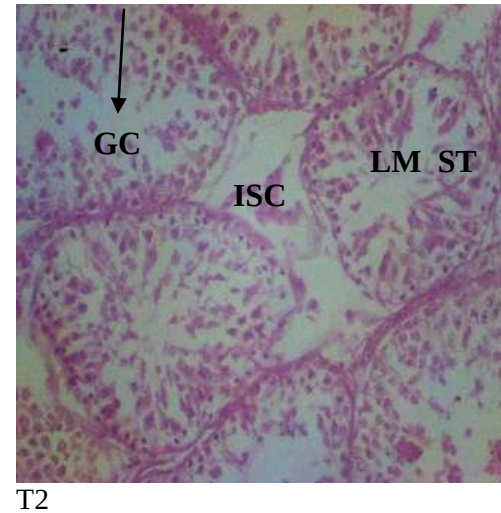
Plate 2: Photomicrograph of the liver of rabbit bucks fed diets supplemented with *Nigella sativa*. PT: Portal triad; SSD: Sinusoid; HC: Hepatocyte; T1: Control, 0% *N. sativa*; T2: 0.5% *N. sativa*; T3: 1.0% *N. sativa;* T4: 1.5% *N. sativa*.

Histological structure of the testis of rabbit buck fed varying levels of Nigella sativa

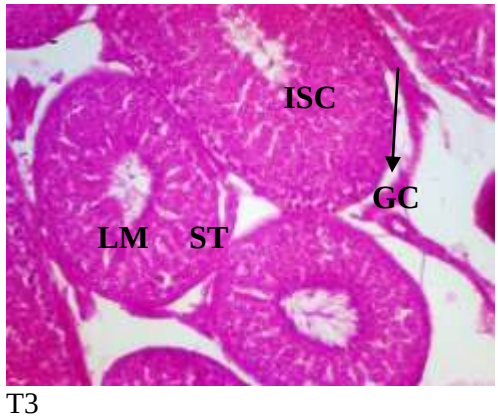
The histological structures of testes of rabbits fed diets supplemented with *Nigella sativa* are shown in Plate 3. In the control rabbits (T1), germ cell population and arrangement within the seminiferous tubules appeared normal but fewer in number compared to the rest of the treatments. Seminiferous tubules with numerous and closely packed (compact) spermatogenic cells were however observed in testes of bucks in T3 and T4. The germ cells were orderly arranged within the seminiferous tubules. The lumina were distinct and contained maturing spermatids. The interstitium contained interstitial cells and blood vessels. The testicular histo-architecture remained normal as *Nigella sativa* supplementation level increased to 1.5%.



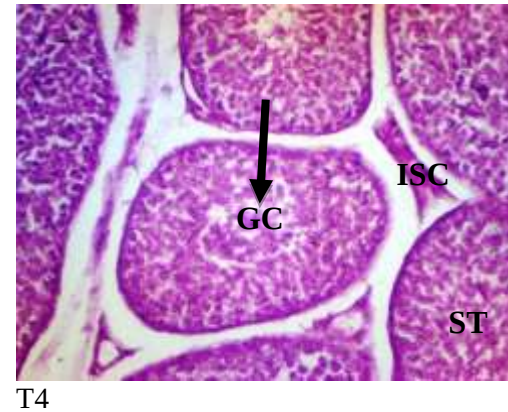
T1



T2



T3



T4

Plate 3: Photomicrograph of the testis of rabbit bucks fed diets supplemented with *Nigella sativa*. GC: Germ cell; LM: Lumen; ISC: Interstitial cells; ST: Seminiferous tubule; T1: Control, 0% *N. sativa*; T2: 0.5% *N. sativa*; T3: 1.0% *N. sativa;* T4: 1.5% *N. sativa*.

Discussion

The photomicrographs of kidney of rabbits fed *Nigella sativa* supplemented diets showed no tissue damage up to 1.0% supplementation level. This observation might be as a result of nephron-protective effect of *Nigella sativa* (Al-Naggar *et al.,* 2003). *Nigella sativa* prevents renal dysfunctions and morphological abnormalities (Al-Naggar *et al.,* 2003). This is achieved by preventing kidney tissues against free radicals (Bendary, 2003). However, signs of mild tubulorrhexis was observed at 1.5% *Nigella sativa* supplementation. This supplementation level could probably be a bit high to cause a mild adverse effect (Dollah *et al*., 2013). Moreover, thymoquinone toxicity has been reported by Badary *et al*. (2000) especially when administered at high dose (Mashayekhi-sardoo *et al*., 2020).

The observation that there was no gross tissue damage in liver histological structure suggests that *Nigella sativa* could exert hepatoprotective effects on rabbit buck's liver. This observation might be due to the protective role of thymoquinone and its derivatives (thymohydroquinone, dithymoquinone, thymol, and carvacrol) which are active components of *Nigella sativa* seed (Eid *et al*., 2017). Mohideen *et al.* (2003) reported that aqueous extract of *N. sativa* seeds showed significant hepatoprotective activity in male Wister rats. The protective role of vitamin E and flavonoids of *Nigella sativa* seed have shown that they are effective in hepatoprotective activities (Al-Okaily *et al*., 2012). However, the mild disruption observed in the liver sinusoids of rabbits fed 1.5% *Nigella sativa* supplementation is an indication that this level is possibly high to have exerted certain degree cytotoxic effect (Dollah *et al*., 2013). Furthermore, black seed contains two compounds that can further exert cytotoxicity when offered to animals at a relatively high dose. These two compounds were found to be terpenoids, one of which showed the presence of carbonyl functionality while the other showed the presence of both carbonyl and hydroxyl functionalities (Dollah *et al*., 2013).

This observation is in line with the report of Mashayekhi-sardoo *et al*. (2020) who reported the *in-vivo* toxicological profile of thymoquinone. These authors inferred that the degree and pattern of toxicity of thymoquinone depend on the dose size, route of administration, duration of exposure and model animal. Furthermore, in a toxicological study, acute oral toxicity of thymoquinone component of the volatile oil of *Nigella sativa* has also been reported by Badary *et al*. (2000)

Testis mainly consists of the seminiferous tubules in which spermatogenesis takes place, and the space between the tubules, the interstitium which contains the Leydig cells and has, among others, an endocrine function (Renkawitz-Pohl *et al*., 2005). In the present study, improved histological changes were observed in the testes of rabbits as *Nigella sativa* supplementation increased up to 1.5%. The improvement in testicular structure and non-deviation from the normal architecture of the control group (T1) confirmed the efficacy of *Nigella sativa* in enhancing male reproduction. The numerous and closely packed germ cells observed at 1.0 and 1.5% supplementation level suggest the spermatogenic potential of black seed. This observation might be influenced by the fatty acid and thymoquinone contents of the seeds as reported by Zanouny *et al*. (2013). The findings support the report of Sultan *et al*. (2014) who opined that thymoquinone has protective effect on testicular parameters. Also, Mahdavi *et al*. (2015) in a systematic review on the effects of black seed and

thymoquinone on male infertility concluded that black seed could exert positive effects on testicular parameters and reproductive organs.

The present study further agrees with the work of Ashraf *et al*. (2013) who reported that oral administration of black seed oil positively influenced histological parameters of testes by increasing the seminiferous tubule sizes, germinal and interstitial cells when compared with the control group. Furthermore, light microscope studies have revealed that *Nigella sativa* treated groups had an increased number of germ and interstitial cells relative to the control group in rats.

Conclusion

In conclusion, findings from this study indicate that *Nigella sativa* seeds possess nutritive and cyto-protective potentials at supplemental level up to 1.5% in rabbit buck diets which preserved and improved the histological architecture of the kidney, liver and testis. Thus, supplementing diets with *Nigella sativa* up to 1.5% could serve as a dietary strategy for improvement and preservation of the structural integrity of kidney, liver and testis of rabbit buck.

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