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Benefits of Adding Black Seed Oil as A Natural Antioxidant in The Diet of Male Adult Rabbits on Biological Body Functions, Oxidative Stress Biomarkers, and Semen Quality

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Abstract: In a four-month experiment, the objective of this study was to determine how adding black seed oil to the diet of mature male rabbits would affect their body's physiological processes and the quality of their semen. In this investigation, twenty-four adult rabbit bucks aged 6 months with an average body weight of 2475.5 g throughout the first production season were used. Bucks were randomly distributed to each of the two experimental groups, according to a final randomized plan. Bucks served as the control group in the first group, and they were fed a commercial pelleted diet without even any supplements. Each buck in the second group received the daily feed requirement from commercial pellets combined with five ml of black seed oil. Every month, measurements of weight gain, feed consumption, testosterone hormone; blood constituents, oxidative stress biomarkers, libido, and semen quality were taken. The findings demonstrated that including black seed oil in the diet of Buck's rabbits improved their daily feed intake, daily weight gain, testosterone hormone, γ-globulin, HDL, and oxidative stress biomarkers, and significantly decreased their glucose, total cholesterol, and LDL levels when compared to the control group. The reaction times and quality semen profile values of the rabbit bucks were also improved significantly by the addition of black seed oil. It was determined that including black seed oil in the diet of male rabbits improved their physiological body processes, caused hypercholesterolemia, and boosted the quality of their sperm without negatively affecting their liver and kidney functions.

Keywords: *Rabbits; Black Seed Oil; Testosterone; Cholesterol; Semen Quality.*

INTRODUCTION

Most nations throughout the world suffer from a meat shortage, and rabbits are crucial to addressing this issue. This significant significance is attributable to rabbits' high reproductive potential, rapid development rate, short generation interval, a wide choice of dietary options, constrained vital areas, and ease of upbringing (El-Saidy *et al.,* 2016; Habeeb *et al.*, 2019). Due to a lack of sufficient feed for rabbit production, various efforts have been made to use feed spices as an addition to animal feed to increase the animals' nutritional content, growth rate, and feed efficiency while also lowering feeding expenses (El-Nomeary *et al.,* 2016).

Every oxidative metabolism produces oxygen-free radical species (ROS), which have the potential to kill an organism's cells. It can be dangerous for animals when ROS is overproduced in their bodies (Krapfenbauer *et al.*, 2003). Reactive oxygen species (ROS) are produced more often under conditions of oxidative stress than they can be safely neutralized by the body's endogenous antioxidant defenses (Hady *et al.*, 2018). Antioxidants are complexes that are used to reduce the generation of free radicals in the body by inhibiting oxidation and scavenging free radicals and reactive oxygen species (ROS).

Natural antioxidants are abundant in medicinal plants. Exogenous antioxidants, in particular, must be added to the animal body to prevent excessive ROS formation and combat free radicals during oxidative metabolism (Vercruysse *et al.*, 2009). By removing the negative effects of oxidative stress and reducing free radicals or ROS in rabbits, the addition of antioxidants to the diet, such as black seed oil, has the potential to improve the health and productivity of farm animals (El-Shanty, 2003; El-Desoky *et al.*, 2017). Black seed oil (Nigella sativa oil) has antioxidant properties because it contains the antioxidant thymoquinone, which purifies harmful free radical scavenging activity and helps rabbits maintain their body homeostasis by enhancing endogenous cellular defense mechanisms to deal with oxidative stress (El-Bagir *et al.*, 2010; Alenzi *et al.*, 2013; Akbarian *et al.*, 2016). The constituents of the black seeds are oil (27–40%), proteins (16–31.2%), carbohydrates (24–40%), crude fiber (5.–8.9%), and minerals (1.79–4.8%) according to Rashid *et al.* (2021). Black seed is a good source of fatty acids, especially, the unsaturated and essential fatty acids that animals cannot produce on their own and must

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therefore obtain from food. Black seed is also rich in monosaccharaides and non-starch polysaccharide components, which is a useful source of dietary fiber (Lutterodt *et al.*, 2010). The predominant fatty acids in the fixed oil were linoleic acid (55.6%), oleic acid (23.4%), and palmitic acid (12.5%), with the levels varying depending on the source which activated the immune system of rabbits when black cumin seeds were given to animals (Ustun-Argon and Gokyer, 2016). Glutamic acid, phenylalanine, lysine, threonine, arginine, aspartic acid, histidine, leucine, isoleucine, glycine, methionine, and valine are the main amino acids in the black cumin seeds (Ustun-Argon *et al.*, 2022). Researchers also identified riboflavin, vitamin A, pyridoxine, thiamin, niacin and vitamin C as the vitamins present in the seeds (Bashir *et al.*, 2021).

The seeds contain between 0.4 and 1.5% essential oil, which is made up of 40 distinct components and the volatile oil's main constituents were transanethole (38.3%), p-cymene (14.8%), limonene (4.3%), carvone (4.0%) methoxypropyl-5-methyl-1, 4-benzenediol, thymol, and carvacrol (Nickavar *et al.*, 2003; Hannan *et al.*, 2021). The oil also contains sterol components and the tocopherols (α, β, and γ). The most prevalent ingredients in essential oils include thymoquinone, thymohydroquinone, dithymoquinone (nigellone), p-cymene, thymol, limonene, carvone, and carvacrol (Kooti *et al.*, 2016). Thymoquinone is the primary bioactive component of the essential oil which is responsible for the majority of the therapeutic actions of Nigella sativa (Tavakkolia *et al.*, 2017).

The purpose of the study is to assess how black seed oil affects mature buck rabbits body biological processes and the quality of their semen.

MATERIALS AND METHODS

Experimental Site

This research was carried out at the Rabbits Farm, which is part of the Atomic Energy Authority's Experimental Farms Project, in Inshas, Egypt (latitude 31o 12' N to 22o 2' N, longitude 25o 53' E to 35o 53' E) during the winter season (January, February, March, and April of 2022). The average of environmental temperature and relative humidity during experimental period were 22±5 ^{O}C and 55±2.0%, respectively.

Experimental Ethics

The Egyptian Atomic Energy Authority's animal care and welfare committee approved the study. Ethical clarification for animal research post conduction No 4PA/22 at 26-12-2022. This section discusses relevant data regarding efforts to lessen animal suffering and adherence to the strictest veterinary precautions.

Feeding, Housing, and Management of Rabbits

Throughout the trial, the two experimental groups of buck rabbits had the same diet. Clover hay (40%), wheat bran (25%), yellow maize (15%), soybeans (10%), molasses (5%), bone meal (2%), calcium carbonate (1%), salt chloride (1%), vitamins & minerals premix (0.5%), and DL-methionine (0.5%) are the ingredients of the marketable food. The marketable food's chemical analysis shows that the DM percentages of crude protein, crude fiber, ether extract, nitrogen-free extract, and ash are respectively 18.5, 12.5, 3.5, 56, and 9.5%. A further 2600 kcal/kg DM of digestible energy is present. Each buck was housed in a galvanized metal hutch that measured 60 by 60 by 45 cm and was 100 cm above the ground. Before the bucks were transferred, the hutches were scrubbed, cleaned, and left for a week to dry. A nipple for drinking and a feeder for feeding the sellable food were also provided for each cage. All experimental groups received free access to food and water. Feces and urine are released from pens and cleaned every day. Clostridium enterotoxaemia bloat was used to safeguard the rabbits before the experiment.

Experimental Design

In this study, twenty-four adult New Zealand White bucks aged 6 months with a live body weight of an average of 2475.5 g in the first production year were used. After randomization, the bucks were carelessly divided into two groups with nine rabbits each. Bucks served as the control group in the first group and were fed commercial pelleted food without any supplements. For four months beginning on first of January to the end of April 2022, each buck in the second group received the daily feed requirement from commercial pellets mixed with five ml of black seed oil/rabbit/day mixed with feed. For four months, the chosen dose of black seed oil was given to those who needed it for their commercial diet. 250 ml of organic black seed oil (SEKEM, pure 100%) was obtained from a neighborhood market in Zagazig city in Egypt. Before the trial began, which lasted four months, the experimental rabbits were in good health and were clinically free of both internal and external parasites. Galvanized wire battery cages measuring 60 by 60 by 45 cm were used to house the animals individually. A feeder and an automatic nipple drinker were included in each cage. Every day, cages were cleaned of urine and waste. All animals were cared for and managed following acknowledged guidelines for the humane treatment of animals, and they were all kept in the same management and sanitary settings. Every two weeks, each buck was weighed. Every one month, an individual's feed intake was estimated.

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Estimation of Blood Biochemical Components and Oxidative Stress Biomarkers:

Every one month during the four-month trial period, one blood sample was taken from each buck. Each animal had five mL of blood drawn from the vein in its ear into a fresh, sterile tube without the use of anticoagulants. Blood samples were allowed to coagulate before being centrifuged at 3000 rounds per minute for 25 minutes to obtain the serum (the supernatant). Up until the time of the analysis of the blood's biochemical components and testosterone concentrations, serum was kept frozen at a temperature of –20°C. Every month, the serum was examined using commercial chemical reagent kits for serum total proteins, albumin, γ-globulin, glucose, total cholesterol, HDL and LDL concentrations, liver function (ALT & AST enzyme activity), and renal function (urea and creatinine levels) (Diamond Diagnostic Company, Egypt). Using diagnostic tools provided by the Bio Diagnostic ® Company, Egypt, oxidative stress biomarkers in rabbit serum were quantified according to the manufacturer's instructions. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH) are two biomarkers for oxidative stress. Every two weeks, radioimmunoassay (RIA) was used to measure the hormonal level of testosterone using a commercially available kit. Iodine-125 was used to mark the testosterone hormone's tracer (I^{125}). After the incubation period, the fluid components in the tubes are evacuated, and the radioactivity of the labeled iodine is measured by a computerized gamma counter at the Egyptian Atomic Energy Authority, Biological Applications Department.

Semen Collection and Assessment of Semen Quality:

The experimental bucks were trained for semen release and collection via an artificial vagina while attempting to mount four mature females as a teaser for one week before the semen collection. The experimental bucks were given a week to become acclimated to the semen collection. During the experimental trial, a single semen sample was taken from each buck as the first ejaculate every month by an artificial vagina employing a female teaser rabbit. A white Vaseline lubricant was used to lubricate the inner rubber sleeve of the prosthetic vagina, which had its temperature set at 40° C. At 10 a.m., we saw the collection of sperm, and the gel was measured and removed right away after semen collection. Using a stopwatch, the reaction time (measured in seconds) from the moment the teaser (a female rabbit) was presented to the buck until the buck jumped and ejaculated the first copulation was used to measure libido (sexual desire or reaction time). All semen samples were maintained in a water bath (35–37 °C) and brought right away to the lab to determine the semen quality. The amount of ejaculate produced was calculated using a graded collection tube attached to the artificial vagina, and the amount of ejaculate produced (in milliliters) was quantified after the gel mass was removed. Using a light microscope with a heated stage and visual examination at (100X) magnifications, the percentage of sperm motility was calculated right after semen collection in numerous microscopic fields and ranged from 0 to 100%. Scores for semen mass motility ranged from 0 to 3. To determine the sperm viability percentage, a dried smear of a drop of each semen ejaculate stained with an eosin-nigrosin blue staining solution was arranged (live or dead). According to the staining outline, the sperm cells were divided into stained (dead sperm) and non-stained categories (live sperm). The percentage of sperm abnormalities was assessed while counting 200 sperm cells and examining the live/dead sperm percentage under a high-power (400X) microscope. Following semen dilution, the enhanced Neubauer hem-cytometer slide (Hamburg, Germany) was used to measure the concentration of sperm cells (10^6/ml) (1:100). By dividing the amount of ejaculation by the sperm concentration and the normal sperm count, the total sperm output (x10^6/ejaculate) was computed.

Statistical Analysis

Data were exposed to the analysis of variance using the general linear model procedure of SAS (2002). The statistical procedure as follows: $Y_{ij} = \mu + T_i + S_j + e_{ij}$ where Y_{ij}= the observation, μ = overall mean, T_i = the fixed effects of treatment (1=control, and 2=black seed oil), S_j= the fixed effects of sampling time (1^{st}, 2^{nd}, 3rd and 4^{th} months) and e_{ij} = random error. Differences with *P* values of <0.05 were accepted significantly. Duncan's Multiple Range Test was used for testing the significant differences between means (Duncan, 1955). Using numerous Z-tests to parallel equivalent amounts, the Chi-square test was used to determine whether the percentages of semen viability and motility were possible.

RESULTS

The Effect of Black Seed Oil on Live Body Weight and Feed Intake

Table (1) provides data about the impact of black seed oil on average body weight, total increase, and average daily growth. After the first, second, third, and fourth months, respectively, the average live body weight increased progressively by 200, 405, 590, and 792 g as a response to the black seed oil treatments. Black seed oil administration also increased significantly the overall weight growth (g/120 days) and average daily gain (g/daily) by 787 and 6.56 g, respectively. Table (1) provides also information on how black seed oil affects daily feed intake and total feed intake (g/120 days). Following treatment with black seed oil, daily feed intake improved during the first, second, third, and fourth months by 15.5, 20.5, 20.0, and 25.0 %, respectively. Furthermore, by treating with black seed oil by 2400 and 20.0 g, respectively, the total feed intake and average feed intake (g/daily) were significantly improved.

Table 1. Effect of adding black seeds oil to the diet of mature male rabbits on live body weight (LBW) and feed intake (FI)

Live body weight (LBW)	Control group	Black seed oil treated group	Monthly increase in BW (g)	P values & significant
No of bucks	12	12	--	---
Initial live body weight (LBW) , g	2475±22	2480±23	--	P>0.05
LBW after the first month, g	2850^b±25	3050^a±33	+200	P<0.01
LBW after the second month, g	3215^b±33	3620^a±37	+405	P<0.01
LBW after the third month, g	3600^b±23	4190^a±24	+590	P<0.01
LBW after the fourth month, g	3998^b±29	4790^a±28	+792	P<0.01
Total gain, g/120 days	1523^b±11	2310^a±18	+787	P<0.01
Average daily gain, g/daily	12.69^b±0.2	19.25^a±0.4	+6.56	P<0.01
Daily FI after the first month , g	150.0^b±8	165.5^a±10	+15.5	P<0.05
Daily FI after the first month , g	165.0^b±12	185.5^a±12	+20.5	P<0.05
Daily FI after the first month , g	175.0^b±15	195.0^a±14	+20.0	P<0.05
Daily FI after the first month , g	180.0^b±13	205.0^a±21	+25.0	P<0.05
Total FI, g/120 days	20400^b±32	22800^a±23	+2400	P<0.05
Average FI, g/daily	170.0^b±15	190.0^a±19	+20.00	P<0.05

a, b. Means in the same row with different superscript differ significantly (P<0.05) or (P<0.01).

The Effect of Black Seed Oil on Blood Protein Fractions

The acquired results for the effect of black seed oil supplementation on blood protein fractions are reported in Table (2). After 1st, 2nd, 3rd, and 4th months, respectively, the serum total protein in the group receiving black seed oil supplements dropped considerably by 15.16, 13.26, 11.42, and 14.77%. Moreover, the mean total protein was lower overall as a result of the treatment (6.27 g/dl) compared to the control (7.17 g/dl), with a loss of approximately 12.55%. This decrease in total protein was consistent with an overall mean decrease of 25.98% in albumin concentration and significant drops in albumin values of 27.11, 26.74, 25.98, and 24.50% after 1st, 2nd, 3rd, and 4th months, respectively. Conversely, black seed oil treatment increased the values of γ-globulin. Compared to control, the overall mean increased in γ-globulin by 23.68%. The best improvement in γ-globulin was seen at fourth month into the treatment, when it increased by 29.73, followed by improvements of 29.46, 23.89, and 21.01% after 1st month, 3rd, and 2nd month, respectively (Table 2).

Table 2. Effect of adding black seed oil on blood protein fractions

Protein fractions	Experimental months	Control group	Black seeds oil treated group	Change, %	P values & significant
Total protein (g/dl)	1st month	7.39^a±0.08	6.27^b±0.27	-15.16	P<0.05
	2nd month	7.09^a±0.01	6.15^b±0.39	-13.26	P<0.05
	3rd month	7.09^a±0.14	6.28^b±0.09	-11.42	P<0.05
	4th month	7.11^a±0.06	6.06^b±0.03	-14.77	P<0.05
	Overall mean	7.17^a±0.01	6.27^b±0.08	-12..55	
Albumin (g/dl)	1st month	4.61^a±0.03	3.36^b±0.10	-27.11	P<0.01
	2nd month	4.60^a±0.17	3.37^b±0.03	-26.74	P<0.01
	3rd month	4.58^a±0.07	3.39^b±0.09	-25.98	P<0.01
	4th month	4.53^a±0.28	3.42^b±0.08	-24.50	P<0.01
	Overall mean	4.58^a±0.02	3.39^b±0.01	-25.98	
γ- globulin (g/dl)	1st month	1.12^b±0.06	1.45^a±0.06	+29.46	P<0.01
	2nd month	1.19^b±0.04	1.44^a±0.06	+21.01	P<0.01
	3rd month	1.13^b±0.03	1.40^a±0.06	+23.89	P<0.01
	4th month	1.11^b±0.02	1.44^a±0.03	+29.73	P<0.01
	Overall mean	1.14^b±0.01	1.41^a±0.01	+23.68	

a, b. Means in the same row with different superscript differ significantly (P<0.05) or (P<0.01).

The Effect of Black Seed Oil on Blood Cholesterol Fractions

The impact of black seed oil on the cholesterol fractions in mature male rabbits was shown in Table (3). The total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels are ameliorated by black seed oil. After 1st, 2nd, 3rd and 4th months, the total cholesterol level has dramatically dropped by 15.84, 13.55, 11.14, and 10.04%, respectively.

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Table 3. Effect of adding black seed oil on blood cholesterol fractions

Cholesterol fractions	Experimental months	Control Group	Black seeds oil treated group	Change, %	P values & significant
Total cholesterol (mg/dl)	1st month	95.84^{a}±2.05	80.66^{b}±3.77	-15.84	P<0.05
	2nd month	92.84^{a}±2.98	80.26^{b}±4.17	-13.55	P<0.05
	3rd month	93.38^{a}±2.18	82.98^{b}±4.03	-11.14	P<0.05
	4th month	93.07^{a}±2.59	83.73^{b}±0.88	-10.04	P<0.05
	Overall mean	93.78^{a}±0.11	81.91^{b}±0.80	-12.66	
LDL (mg/dl)	1st month	58.59^{a}±0.90	42.30^{b}±1.05	-27.80	P<0.01
	2nd month	59.00^{a}±0.64	41.07^{b}±0.58	-30.39	P<0.01
	3rd month	60.15^{a}±0.50	42.20^{b}±0.77	-29.84	P<0.01
	4th month	59.66^{a}±0.53	41.50^{b}±0.53	-30.44	P<0.01
	Overall mean	59.35^{a}±0.17	41.77^{b}±0.25	-29.62	
HDL (mg/dl)	1st month	28.38^{b}±0.88	36.15^{a}±0.18	+27.38	P<0.01
	2nd month	28.39^{b}±0.31	35.44^{a}±0.26	+24.83	P<0.01
	3rd month	29.30^{b}±0.14	38.81^{a}±0.44	+32.46	P<0.01
	4th month	29.36^{b}±0.42	36.87^{a}±0.47	+25.58	P<0.01
	Overall mean	28.86^{b}±0.02	36.82^{a}±0.02	+27.58	

a, b. Means in the same row with different superscript differ significantly (P<0.05) or (P<0.01).

Additionally, the LDL dropped by 27.80, 30.39, 29.84, and 30.44% throughout 1st, 2nd, 3rd, and 4th months with a mean reduction overall that was 29.62% lower than the control. On the other hand, a beneficial increase in serum HDL was seen after first month by 27.38%, second month by 24.83%, third month by 32.46%, and forth month of treatment by 25.58%, with a mean change percentage increase of 27.58 overall (Table 3).

The Effect of Black Seed Oil on Kidney and Liver Functions

Regarding kidney function, the treatment had the desired effect of considerably reducing serum urea and creatinine levels at all study periods. Urea content decreased throughout the study from the first month to the forth month experiment, following a 21.23% fall in the overall mean. In connection to supplementing with black seed oil, serum creatinine also fell. After eight weeks of treatment, a significant decline was noted by 36.36%, and by six weeks, a significant decrease was noted by 34.62% (Table 4).

Table 4. Effect of adding black seed oil on kidney and liver functions

Kidney and liver functions	Experimental months	Control group	Black seeds oil treated group	Change, %	P values & significant
Urea (mg/dl)	1st month	53.45^{a}±1.33	43.91^{b}±0.53	-7.85	P<0.05
	2nd month	58.76^{a}±1.14	44.66^{b}±1.97	-24.00	P<0.01
	3rd month	56.47^{a}±2.09	44.25^{b}±2.75	-21.64	P<0.01
	4th month	56.68^{a}±0.68	44.69^{b}±0.80	-21.15	P<0.01
	Overall mean	56.34^{a}±0.07	44.38^{b}±0.16	-21.23	
Creatinine (mg/dl)	1st month	1.32^{a}±0.06	0.86^{b}±0.02	-34.85	P<0.01
	2nd month	1.32^{a}±0.06	0.85^{b}±0.03	-35.61	P<0.01
	3rd month	1.30^{a}±0.02	0.85^{b}±0.09	-34.62	P<0.01
	4th month	1.32^{a}±0.04	0.84^{b}±0.10	-36.36	P<0.01
	Overall mean	1.32^{a}±0.01	0.85^{b}±0.01	-35.61	
ALT (U/l)	1st month	42.51^{a}±1.95	33.89^{b}±2.93	-20.28	P<0.01
	2nd month	44.55^{a}±0.63	33.26^{b}±1.71	-25.34	P<0.01
	3rd month	41.32^{a}±1.38	33.07^{b}±1.72	-19.97	P<0.01
	4th month	40.66^{a}±1.09	34.29^{b}±0.59	-15.67	P<0.01
	Overall mean	42.26^{a}±0.23	33.63^{b}±0.43	-20.42	
AST (U/l)	1st month	39.32^{a}±3.18	25.11^{b}±1.88	-36.14	P<0.01
	2nd month	39.57^{a}±0.84	28.47^{b}±1.27	-28.05	P<0.01
	3rd month	38.83^{a}±0.56	29.57^{b}±0.30	-23.85	P<0.01
	4th month	38.41^{a}±0.39	29.59^{b}±0.60	-22.96	P<0.01
	Overall mean	39.03^{a}±0.15	28.19^{b}±0.01	-27.77	

a, b. Means in the same row with different superscript differ significantly (P<0.05) or (P<0.01).

According to Table (4), liver enzyme activity (ALT, AST) significantly decreased after black seed oil treatment. Due to the black seed oil treatment, ALT was dramatically reduced, with a 20.42% total mean decrease. Table clarifies information on AST activity that was treated with black seed oil. AST activity was seen to dramatically decrease after first month of treatment, then again after second, third and fourth months, with the greatest decrease occurring after four months. Over the control, the total mean dropped by 27.77%.

The Effect of Black Seed Oil on Blood Serum Antioxidant Enzyme Activity and Testosterone Hormone and Glucose

Table (5) provides information demonstrating black seed oil's impact on serum antioxidant activity. At every stage of the experiment, black seed oil administration resulted in appreciable increases in the activity of the enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD).

Table 5. Effect of adding black seed oil serum antioxidant enzymes activity and concentrations of testosterone hormone and glucose in buck rabbits

Antioxidant enzymes	Experimental months	Control group	Black seeds oil treated group	Change, %	P values & significant
Glutathione peroxidase (GSH), µmol/dl	1st month	$25.00^{b}\pm0.58$	$29.00^{a}\pm0.58$	+16.00	P<0.05
	2nd month	$25.33^{b}\pm0.67$	$30.33^{a}\pm0.67$	+19.74	P<0.05
	3rd month	$25.33^{b}\pm0.33$	$31.33^{a}\pm0.67$	+23.69	P<0.01
	4th month	$26.00^{b}\pm0.58$	$32.00^{a}\pm0.58$	+23.08	P<0.01
	Overall mean	$25.42^{b}\pm021$	$30.67^{a}\pm0.65$	+20.65	
Superoxide dismutase (SOD), U/dl	1st month	$11.37^{b}\pm0.69$	$15.27^{a}\pm0.23$	+34.30	P<0.01
	2nd month	$12.17^{b}\pm0.44$	$16.47^{a}\pm0.38$	+35.33	P<0.01
	3rd month	$13.03^{b}\pm0.09$	$17.70^{a}\pm0.29$	+35.84	P<0.01
	4th month	$13.10^{b}\pm0.31$	$17.67^{a}\pm0.39$	+34.89	P<0.01
	Overall mean	$12.42^{b}\pm0.41$	$16.78^{a}\pm0.58$	+35.10	
Testosterone (ng/ml)	1st month	$4.74^{b}\pm0.37$	$7.78^{a}\pm0.42$	+64.14	P<0.01
	2nd month	$5.80^{b}\pm0.29$	$7.69^{a}\pm0.45$	+32.59	P<0.01
	3rd month	$4.22^{b}\pm0.39$	$9.20^{a}\pm0.52$	+118.01	P<0.01
	4th month	$5.01^{b}\pm0.38$	$9.14^{a}\pm0.42$	+82.44	P<0.01
	Overall mean	$4.94^{b}\pm0.28$	$8.45^{a}\pm0.02$	+71.05	
Glucose (mg/dl)	1st month	$75.81^{a}\pm3.21$	$64.47^{b}\pm3.21$	-14.96	P<0.05
	2nd month	$76.43^{a}\pm2.28$	$67.07^{b}\pm2.12$	-12.25	P<0.05
	3rd month	$71.50^{a}\pm2.83$	$62.40^{b}\pm1.85$	-12.73	P<0.05
	4th month	$66.43^{a}\pm2.96$	$57.98^{b}\pm1.18$	-12.72	P<0.05
	Overall mean	$72.54^{a}\pm1.79$	$62.98^{b}\pm1.56$	-13.18	

a, b. Means in the same row with different superscript differ significantly (P<0.05) or (P<0.01).

The expected impact was shown after 3rd month, with subsequent intervals of 4th month, 2nd month, and 1st months. Additionally, black seed oil administration increased SOD activity because the change percentage peaked after 3rd month at 35.84% and peaked again after 2nd month at 35.33%. Data from Table (5) showed that supplementing with black seed oil enhanced the levels of the testosterone hormone during all study weeks. When black seed oil was added, the mean concentration of the testosterone hormone increased dramatically from 4.94 in the control group to 8.45ng/ml in the supplemented group. In the opposite direction, adding black seed oil to the diet of male rabbits considerably reduced the levels of glucose at all of the experiment's intervals, with a fall in the overall mean of 13.18%.

The Effect of Black Seed Oil on The Semen Quality of Male Rabbits

Table (6) contains information on the impact of black seed oil on semen quality. The reaction time was dramatically shortened significantly by 8.14, 10.59, 12.50, and 12.20% due to the addition of black seed oil after 1st, 2nd, 3rd, and 4th months, respectively. Overall, there was a 10.91% decline in the mean reaction time ($second^{-1}$).

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Table 6. Effect of adding black seed oil to the diet of mature male rabbits on semen quality

Semen quality	Experimental months	Control group	Black seeds oil treated group	Change %	P values & significant
Reaction time second^{-1}	1st month	8.60^a±0.01	7.90^b±0.01	-8.14	P<0.05
	2nd month	8.50^a ±0.01	7.60^b±0.01	-10.59	P<0.05
	3rd month	8.40^a±0.01	7.35^b±0.01	-12.50	P<0.05
	4th month	8.20^a±0.01	7.20^b±0.01	-12.20	P<0.05
	Overall mean	8.43^a±0.07	7.51^b±0.05	-10.91	
Ejaculate volume x 10^{-2} ml	1st month	83.67^b±8.68	102.33^a±0.88	+22.30	P<0.01
	2nd month	83.33^b±8.42	116.67^a±3.34	+40.01	P<0.01
	3rd month	95.54^b±4.56	150.22^a±5.45	+57.23	P<0.01
	4th month	106.67^b±3.34	186.67^a±8.83	+75.00	P<0.01
	Overall mean	92.30^b±3.94	138.97^a±12.89	-50.56	
Total motility percentage	1st month	81.67^b±0.88	87.67^a±0.33	+7.35	P>0.05
	2nd month	82.67^b±1.45	90.67^a±0.67	+9.68	P<0.05
	3rd month	82.66^b±0.60	92.58±0.55	+12.00	P<0.05
	4th month	83.33^b±0.88	93.00^a±0.58	+11.60	P<0.05
	Overall mean	82.58^b±0.24	90.98^a±0.15	+10.17	
Progressive sperm motility (PMOT, %)	1st month	68.33^b±0.88	71.67^a±0.88	+4.89	P>0.05
	2nd month	69.67^b±0.33	72.67^a±0.33	+4.31	P>0.05
	3rd month	69.55^b±0.77	85.90^a±0.65	+23.51	P<0.01
	4th month	69.00^b±0.58	93.00^a±0.58	+34.78	P<0.01
	Overall mean	69.14^b±0.19	80.81^a±2.51	+16.88	

a, b. Means in the same row with different superscript differ significantly (P<0.05) or (P<0.01).

Black seed oil increased the ejaculate volume (x10^{-2} ml) in all experimental moths following a rising percentage of the overall mean by 50.56%. Once more, the treatment had a considerable positive impact on the percentage of overall motility. After the end of first month, the experiment's total motility percentage was determined to be significantly increased. Due to the addition of black seed oil, the overall mean total motility percentage increased by 10.17%. As shown in Table (6), no significant effect of black seed oil supplementation on progressive sperm motility in the first two months from the experimental period. After the first two months, black seed oil supplementation had a substantial impact on progressive sperm motility (PMOT percentage). After adding black seed oil at a substantially higher percentage, PMOT% increased as desired. An accepted preferred change of 16.88% was detected in the percentage of the overall mean.

Table (7) provides findings about the impact of black seed oil on the percentage of viable sperm. Live sperm percentage was unaffected by black seed oil supplementation after two months, but it considerably increased by 9.53 and 11.35% after third and fourth months, along with a percentage increase in the overall mean of 5.64%.

Table 7. Effect of adding black seed oil to the diet of mature male rabbits on semen quality

Semen characteristics	Experimental months	Control group	Black seeds oil treated group	Change %	P values & significant
Live sperm (%)	1st month	81.67±0.88	81.67±0.88	0.00	P>0.05
	2nd month	82.67±0.33	84.00±0.58	+1.61	P>0.05
	3rd month	82.66b±0.66	90.54a±0.77	+9.53	P<0.05
	4th month	82.33b±1.20	91.67a±1.20	+11.35	P<0.05
	Overall mean	82.33b±0.12	86.97a±0.40	+5.64	
Dead sperm (%)	1st month	18.33±0.88	18.33±0.88	0.00	P>0.05
	2nd month	17.33a±0.33	16.00b±0.58	-7.67	P<0.05
	3rd month	17.34a±0.66	9.46b±0.77	-45.44	P<0.01
	4th month	17.67a±1.20	8.33b±1.20	-52.86	P<0.01
	Overall mean	17.67a ±0.12	13.03b±0.40	-26.26	
Normality %	1st month	66.67±2.03	66.00±2.08	1.01	P>0.05
	2nd month	67.00±2.08	69.67±0.88	+3.99	P>0.05
	3rd month	67.60b±2.56	72.88a±1.88	+7.81	P<0.05
	4th month	67.00b±2.08	73.67a±0.67	+9.96	P<0.05
	Overall mean	67.07b±0.21	70.56a±0.28	+5.20	
Sperm abnormalities (%)	1st month	33.33±2.03	34.00±2.08	+2.01	P>0.05
	2nd month	33.00±2.08	30.33±0.88	-8.09	P>0.05
	3rd month	32.40a±2.65	27.12b±1.88	-16.30	P<0.05
	4th month	33.00a±2.08	26.33b±0.67	-20.21	P<0.05
	Overall mean	32.93a±0.12	29.45b±0.28	-10.57	
Sperm-cell concentration $x10^6$ ml^{-1}	1st month	81.20±0.70	81.67±0.88	+0.58	P>0.05
	2nd month	81.00±0.58	82.67±0.33	+2.06	P>0.05
	3rd month	81.45b±0.69	94.55a±0.54	+16.08	P<0.05
	4th month	81.67b±0.88	95.00a±0.58	+16.32	P<0.05
	Overall mean	81.33b±0.08	88.47a±0.16	+8.78	
Total sperms output ($x10^6$ /ejaculate)	1st month	90.50±0.40	92.33±1.20	+2.02	P>0.05
	2nd month	90.67b±0.67	113.33a±6.67	+24.99	P<0.01
	3rd month	92.55b±4.32	140.55a±5.23	+51.86	P<0.01
	4th month	92.67b±1.45	195.00a±2.89	+110.42	P<0.01
	Overall mean	91.60b±0.04	135.3a±19.25	+47.71	

a, b. Means in the same row with different superscript differ significantly (P<0.05) or (P<0.01).

After the first month of the experiment, black seed oil considerably reduced the percentage of dead sperm in the 3rd and 4th months by 45.44 and 52.86%, respectively. These considerable decreases were consistent with an overall mean decrease of 26.26%. After first and second months, there were further notable increases in the normalcy percentage of 7.81 and 9.96%, respectively, as well as an increase of 5.20% in the average normality percentage. No significant effect of black seed oil supplementation on percentage of sperm abnormalities in the first two months from the experimental period. The mean percentage of sperm abnormalities decreased dramatically during the 3rd and 4th months by 16.3 and 20.21 % and the overall mean as a result of treatment decreased by 10.57%. No significant effect of black seed oil supplementation on the number of sperm cells per 106 ml^{-1}in the first two months from the experimental period. After first and second months, there was a significant increase of 16.08 and 16.32% in the number of sperm cells per 106 ml^{-1}, respectively. Additionally, treatment with black seed oil resulted in an 8.78% rise in the overall mean. After first month of the experiment, the black seed oil treatment significantly raised the total sperm yield ($x10^6$/ejaculate) by 24.99, 51.86, and 110.42%. Moreover, there was a 47.71% increase in the overall mean percentage of the total sperm yield (Table 7).

DISCUSSION

The Effect of Black Seed Oil on Feed Intake and Body Weight of Bucks

Bucks who consumed black seed oil in their diet ate more and put on more weight. Black seed oil treatment increased both the total weight gain and the average daily gain by 787 and 6.56 g, respectively. Following black seed oil treatment, daily feed intake increased by 15.5, 20.5, 20.0, and 20.5 %, during the 1st, 2nd, 3rd and 4th months, respectively. A further benefit of treatment with black seed oil was that it increased feed intake by 2400 g in 120 days and averaged feed intake by 20.00 g daily.

The total and average daily growth and the feed conversion ratio were dramatically increased in bucks fed a diet supplemented with black cumin seed meals for 68 days (El-Nomeary *et al.*, 2015). According to Mehrez *et al.* (2011), groups given Nigella sativa meal had the greatest values for body weight, body weight increase, and economic efficiency. Zeweil *et al.* (2008) reported

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that rabbits fed 12% Nigella sativa food had significantly greater overall weight gain values than control, which was 7.7% higher. Supplementing with Nigella sativa meals greatly boosted daily body growth and feed conversion while significantly reducing daily feed intake in rabbits (El-Nomeary *et al.,* 2016). Male rabbits fed black seed oil acquired the most weight when compared to control animals in terms of live weight, overall weight, average daily increase, and feed conversion ratio (Ragab *et al.,* 2016). The best metrics of body mass, weight gain, and economic efficiency are seen in rabbits fed black seed (Mehrez *et al.,* 2011). The consumption of feed and weight gain in rabbits were both significantly influenced by Nigella sativa (Anoh, 2017). A buck's performance appears to be enhanced by adding black seed to its diet, which may be because of the seed's high concentration of active substances like nigellone, thymoquinone, and thymohydroquinon, as well as necessary fatty and amino acids (Abd El-Hakim *et al.,* 2002). Animals' immune systems are aided by the macro-and micro-elements in Nigella sativa, which are in charge of controlling all of the body's essential processes (Seleem and Riad, 2005). Nigella sativa contains the vitamins thiamine, riboflavin, pyridoxine, and niacin, which are crucial for rabbit growth (Seleem *et al.,* 2007). The use of Nigella oils to prevent non-enzymatic peroxidation may improve immunity (Chevallier, 1996).

The Effect of Black Seed Oil on Serum Protein Fractions in Bucks

Serum total protein levels in the group receiving black seed oil supplements dropped significantly and treatment-related mean total protein levels showed a reduction of roughly 12.55%. Albumin levels had a mean reduction of 25.98% overall, which was consistent with a decrease in total protein that was also considerable. Black seed oil treatment improved the Gama-globulin levels, resulting in a 23.68% rise in the overall mean compared to the control.

According to Tousson *et al.* (2011), the feeding meal supplemented with Nigella sativa raised plasma total proteins, albumin, and globulin in the rabbit. In a study conducted by Umar *et al.* (2018), male rabbits were given black seed oil orally at 5ml/kg body weight/day for 60 days and found that substantial rise in plasma total proteins, albumin, and globulin. From this perspective, adding black seed oil to the diet enhanced total protein (Mohammad, 2015). The protein in black seeds is composed of fifteen amino acids, including eight of the nine necessary amino acids. The majority of trace elements, including calcium, iron, sodium, and potassium, are also found in black seeds and serve as vital cofactors for a variety of enzyme functions (Habeeb and El-tarabany, 2012). Due to its low cost compared to other protein sources, nigella seeds are a good source of protein for farm animals to consume (30% or more). As a result, black seed can be used as a good source of protein and energy for animal nutrition because it is a rich source of crude protein and fat (Habeeb and El-Tarabany, 2012).

The Effect of Black Seed Oil on Cholesterol Fraction Levels in Bucks

The addition of black seed oil to the diets of bucks had a substantial impact on serum cholesterol fractions. It was discovered that including nigella sativa oil in the food of bucks decreased their levels of total cholesterol and LDL cholesterol while raising their levels of HDL cholesterol.

According to Tousson *et al.* (2011), the feeding meal supplemented with Nigella sativa lowering total lipids, cholesterol, and triglycerides in rabbit. In a study conducted by Umar *et al.* (2018), male rabbits were given black seed oil orally at 5 ml/kg body weight/day for 60 days decline total lipids, triglycerides, and cholesterol. From this perspective, adding black seed oil to the diet lowering plasma cholesterol and triglycerides. By considerably lowering the level of low density lipoprotein cholesterol, Nigella sativa has an antiatherogenic impact. After using N. sativa for 28 days, LDL cholesterol dramatically dropped, but HDL cholesterol significantly increased (Mohammad, 2015). After therapy for 2, 4, 6, and 8 weeks, N. sativa was found to significantly lower total cholesterol, low-density lipoprotein cholesterol, and increase high-density lipoprotein cholesterol (HDL) levels in comparison to the positive control group (Mohammad, 2015). El-Nomeary *et al.* (2016) found that when compared to rabbits fed a control diet; those fed black cumin seed meals had considerably lower total cholesterol, HDL, and LDL concentrations. According to a study by Pourghassem-Gargari *et al.* (2009), hyperlipidemic rabbits given black seeds demonstrated a considerable decrease in serum total cholesterol and LDL levels and an increase in HDL concentrations. Additionally, it was shown that supplementing a feeding diet with black seeds decreased total lipids, cholesterol, and triglycerides. Black seed is a fantastic source of omega-3 fatty acids in addition to being a great source of protein and sterols. The oil from Nigella seeds contains antioxidant-sitosterol, which inhibits the absorption of cholesterol from the diet (Mehrez *et al.,* 2011). The synergistic activity of specific components, such as thymoquinone and nigellamine, may be to blame for the hypolipidemic impact of black seed oil (Ali and Blunden, 2003). The oil contains a lot of β-sitosterol, which prevents the body from absorbing dietary cholesterol (Atta, 2003).

The Effect of Black Seed Oil on Liver And Kidney Functions of Bucks

Regarding kidney function, the therapy had the expected effect of dramatically reducing serum creatinine and urea levels at all experiment intervals. The use of black seed oil significantly decreased the activity of liver enzymes (ALT, AST). Due to the black seed oil treatment, ALT was dramatically reduced, with a 20.42% total mean decrease. In comparison to the control, AST activity dramatically decreased, and the total mean fell by 27.74%.

Our findings conflict with those of Omar (2003), who discovered a significant rise in serum ALT and AST activity when rabbit diets were supplemented with Nigella Sativa and suggested that the quantity and quality of protein in the diet may affect ALT and AST activity. Our findings conflict with those of Tousson *et al.* (2011), who discovered that rabbits fed a diet enriched with Nigella Sativa as opposed to a control diet tended to have considerably higher quantities of urea and creatinine in their blood serum. These findings, however, demonstrate that adding Nigella Sativa oil to food has no negative impact on liver and kidney function.

The Effect of Black Seed Oil on The Activity of Antioxidant Enzymes And Testosterone And Glucose Levels in Bucks

At every stage of the experiment, black seed oil administration resulted in appreciable increases in the activity of the enzymes glutathione peroxidase and superoxide dismutase. Thymoquinone, cavrvacrol, p-cymene, and 4-terpinol are a few examples of antioxidants found in black seed oil that are thought to be responsible for the substance's medicinal effects in male rabbits given black seed oil orally at 5ml/kg body weight/day for 60 days (Umar *et al.,* 2018). Nigella sativa seeds significantly boosted catalase activity from 3.85 to 4.87 mmol/l and total antioxidant capacity from 0.864 to 1.31 mmol/l in male rats (Mohmoud *et al.,* 2021). According to Ardiana *et al.* (2020), supplementing humans with nigella sativa does not significantly affect level of total antioxidant capacity, although it may be advantageous as an antioxidant by raising superoxide dismutase levels. The production and activity of antioxidant enzymes including glutathione peroxidase, catalase, superoxide dismutase, and glutathione can be increased by nigella sativa (Kazemi, 2014).

Concern of testosterone, results from our study showed that the testosterone hormone concentration was greatly improved by the addition of black seed oil during all experimental weeks. By including black seed oil in the diet of male rabbits, glucose levels were dramatically reduced, with a decrease in the overall mean of 13.18%.

In a study conducted by Umar *et al.* (2018), male rabbits were given black seed oil orally at 5 ml/kg body weight/day for 60 days found that the treated group showed a significant increase in the hormone levels FSH, LH, and testosterone. According to the findings of our study, black seed oil improves the prostate's ability to produce more testosterone, which gives male rabbits the strength and endurance needed to complete the sexual process. When compared to control rabbits, animals given black seed oil had considerably higher blood plasma testosterone levels (Ragab *et al.,* 2016). Adult rabbit testicular weight and length, circumference, and volume, as well as the thickness of the spermatogenic epithelium, the diameter of seminiferous tubules, and the diameter of seminiferous tubule lumen, were significantly increased after oral supplementation with black seed oil (Umar *et al.,* 2017). Testosterone levels were considerably greater in adult rabbits who received black seed oil orally. These results point to a potential function for black seed oil in enhancing testosterone levels through enhancing male reproductive activities (Umar *et al.,* 2017). Oil-treated rabbits showed higher testosterone levels as a result of an impact on the hormone-producing enzyme 17-beta hydroxyl-steroid dehydrogenase. The main component of black seed oil, thymoquinone, has a biological effect on animals that elevates testosterone hormone levels and, thanks to its antioxidant qualities, the guard's wounded testicular tissues (Ayan *et al.,* 2016). Additionally, the larger testicles result in an increase in interstitial/Leydig cells, which raises the level of testosterone (Umar *et al.,* 2017). Our findings conflict with those of El-Nomeary *et al.* (2016), who discovered that rabbits fed black seed, had non-significant alterations in serum glucose, confirming the consistency of the body homeostasis of bucks. Mohammad (2015) reported that sativa oil administration promoted the release of sexual hormones. Mustari *et al.* (2022) found that male mice who received black seed oil at a rate of 0.5 ml/kg had considerably higher levels of testosterone.

The Effect of Black Seed Oil on The Semen Quality of Rabbit Bucks

The addition of black seed oil dramatically decreased the reaction time. The treatment resulted in a 50.56% increase in ejaculate volume (x 10^{-2} ml), exceeding the overall mean percentage. The overall mean total motility percentage improved by 10.17% as a result of treatment, and the total motility percentage greatly increased as a result. After adding black seed oil, PMOT% increased as expected, thanks to a large 16.88% increase in the percentage of the overall mean. The percentage of viable sperm increased dramatically as the total mean percentage increased by 5.64%. The overall mean percentage of dead sperm dropped dramatically by 26.26%. The overall mean percentage of sperm abnormalities decreased dramatically as a result of treatment by 10.57%. Sperm-cell concentration increased significantly as a result of therapy, by 8.78% x 106 ml^{-1} on average. The average number of sperm produced ($x10^6$/ejaculate) increased dramatically by 47.71%. Because black seed oil has antioxidant capabilities, it improved the semen characteristics in this study.

Nigella sativa may be useful in increasing desire and sperm quality in bucks by increasing testosterone levels, which will then increase sexual activity. The black seed oil which contains good antioxidants that protect cells from oxidation and subsequently improve sperm concentration in rabbits, maybe the cause of the improvement in sperm quality and quantity seen in the

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current study (Castellini *et al.*, 1999 and 2003). Antioxidant administration may reduce oxidative stress and enhance sperm motility (Castellini, 2008). Antioxidants have a role in stabilizing the spermatic cell membrane, lipid peroxidation, and sperm DNA oxidation, which are the causes of sterility in male animals and reactive oxygen species (Greco *et al.*, 2005). Nigella sativa oil use also produced the best outcomes in terms of volume, motile sperm percentage, sperm concentration, total sperm per ejaculation, reaction time, and total motile and functional sperm fraction in rabbit males fed diets supplemented with the oil. These improvements in semen characteristics and the decrease in free radicals in the bucks' seminal plasma were seen in the male rabbits (El-Tohamy *et al.*, 2010). Male rabbits fed black seed oil displayed the lowest percentage of sperm abnormalities, the highest levels of sperm motility, viability, and concentration, as well as extremely significant semen volume and gel. When compared to control animals, the group of bucks fed black seed oil reported significantly higher total counts, total motile, totally live, and normal ejaculates (Ragab *et al.*, 2016). According to Darand *et al.* (2020), male mice given black seed oil had significant changes in their sperm, semen, leydig cell count, and testosterone hormone levels, which increased the quality of the semen produced by buck rabbits.

CONCLUSION

According to the study, adding black seed oil to the diet of buck rabbits is a cheap and risk-free strategy that may be advised for usage to increase the semen quality and promote effective growth and bodily functions in mature male rabbits.

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Conflict of Interest

The authors all disclosed that they had no potential conflicts of interest. All authors agreed to withhold any financial advantage or interest they may have had from using our study directly.

Acceptance of Participation

To finish this manuscript, all of the authors contributed. All of the writers of this research report actively contributed to the design, implementation, and analysis of the study. The submitted final version has been read by all writers and received their approval.

Permission To Publish

The work submitted by all authors was accepted for publication. While the paper is being considered by the journal, the content will not be copied, submitted, or published elsewhere. None of the writers of this essay have written any other directly similar works, either published or unpublished. The Egyptian Atomic Energy Authority was consulted before the work was submitted, and all authors explicitly agreed to submit it and get approval.

Availability of Information:

All authors affirmed that the information and resources available support their stated claims and adhere to industry standards.

Declaration And Disclosures:

The study has received approval from the relevant ethics committee for animal research under the Egyptian Atomic Energy Authority and a declaration on the welfare of animals. There are no implications for the general welfare or public health in the material that has been submitted for publication.

Novelty Statement:

Black seed oil in the diet of male rabbits improved their daily feed intake, daily weight gain, testosterone hormone, γ-globulin, HDL, and oxidative stress biomarkers. Black seed oil also significantly decreased their glucose, total cholesterol, and LDL levels. Black seed oil significantly improved the reaction times and quality semen profile values of the rabbit bucks. Black seed oil significantly improved their physiological body processes, caused hypercholesterolemia, and boosted the quality of their sperm without negatively affecting their liver and kidney functions.

The Author's Contribution

- Alsaied Alnaimy Habeeb was responsible for the idea and design of the study's protocol as well as the final approval of the version to be presented.
- Ahmed K. Sharaf collected the data and performed a statistical analysis of the data.
- Mostafa Abbas A. Atta performed the data interpretation and article drafting.
- Anhar Ibrahim Aly Elhanafy critically revised the article and looked for significant intellectual material.

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