# Bioactive Effects of Ginger and Black Seed Oils on Male Rat Fertility

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## ABSTRACT

*This study was carried out to investigate the bioactive effects of ginger and black seed oils on the fertility of male rats. Thirty albino rats of the Sprague-Dawley strain were randomly distributed into 5 groups, the 1st group (6 rats) was fed the basal diet as negative control (-ve). The 2nd group received lead acetate (60mg/kg bw.) orally by gavage three times per week to induce infertility and served as +ve control. Groups 3, 4, and 5 were the same as group 2 but fed on a basal diet containing 5% ginger, 5% black seed oils, 2.5% of each oil, respectively. Fatty acid content and total phenolic content of both oils were estimated. At the end of the experimental period (8 weeks), feed intake, body weight gain, and feed efficiency ratio were calculated. Sex Organs (spermatogenic cells) were collected for histological examination. Lipid profiles, Testosterone, malondialdehyde, and catalase were determined. The results indicated that ginger had the highest value of saturated fatty acids and phenolic content compared to black seed oil but black seeds had higher values of unsaturated fatty acids. Tested oils caused a significant reduction of TC, LDL-c, VLDL-c, and MDA. On the contrary, there was a substantial increase in catalase compared to the +ve control group. It could be concluded that ginger and black seed oils have a potential effect on fertility therefore, this study recommends increasing the dietary intake of ginger and black seed oils because these oils may be beneficial for patients with low fertility.*

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**Keywords:** *ginger oil, black seed oil, fertility.*

## INTRODUCTION

Infertility affects at least one in seven heterosexual couples of reproductive ages, globally. Up to middle age (from 40 years to 65 years old), the incidence of infertility is almost equal in women and men, 35% and 30%, respectively; in 20% of instances mutual factors are involved and in 15% of instances no attribution can be made. For most men with infertility, etiology remains undefined, in part owing to a lack of foundational knowledge around the processes of gamete production and quality and how they are affected by conservational and lifestyle factors. This difficulty is compounded because of the failure of health systems to recognize the burden of male infertility at a population level and its potential as a biomarker of systemic illness **O'Bryan *et al.*, (2024).**

The male reproductive system is highly susceptible to noxious influences, such as oxidative stress, inflammation, drugs, and even diseases that can induce germ cell damage and alterations in spermatogenesis. All of these factors, which are caused by actions at the testicular level and the excurrent ducts and accessory glands, significantly affect sperm parameters and male fertility. For this reason, it is of major importance to investigate possible ways to protect the male reproductive system since males are exposed to these toxic factors constantly **Martins *et al.*, (2021).**

*Nigella sativa* normally known as "black seed" is a flowering plant that grows in countries neighboring the Mediterranean Sea and in India, Iran, and Pakistan. The people of Southeast Asia and Middle Eastern countries have used *N. sativa* seeds to treat disorders, such as asthma, bronchitis, inflammation, infections, and gastrointestinal diseases, and functional oil to treat skin diseases such as eczema and boils. *N. sativa* seeds play a vital role in treating various diseases, especially chronic headaches, fever, hypertension, and migraine (**Mashayekhi-Sardoo et al., 2020).**

The extracts of *N. sativa* are traditionally used as an intestinal antiprotozoal agent, laxative, and carminative. In the past 2 decades, various pharmacological of *N. sativa* including its anti-inflammatory, antioxidant, anti-cancer, immunomodulatory, analgesic, diuretic, antihyper-tensive, antidiabetic, antibacterial, neuro-protective, gastroprotective, and hepatoprotective properties, have been reported. *N. sativa* has been used in medical research for nervous disorders, hyper-lipidemia, hypertension, rheum-atoid arthritis, obesity, lung disease, thyroid dysfunction, hepatitis, and male infertility **(Mahmoud and Abdelrazek 2019).**

Ginger (*Zingiber office-nale*) fits the *Zingiberaceae* family of flowering plants and is a very commonly used herbal spice due to its aromatic and strong flavor. There are hints that the plant was medicinally used already about 5000 years ago, mainly in India and China. The ginger rhizome has traditionally been practical against diseases such as diarrhea, cholera, cold, nausea and hemorrhage, abdominal pain, lumbago, toothache, hypertension, or the chronic inflammatory disease rheumatoid arthritis **Bischoff-Kont and Fürst, (2021).**

The ginger improves semen quality by improving dissimilar sperm parameters mainly count, motility, viability, morphology, and DNA integrity. According to research consequences in various species, ginger appears to have strong antioxidant properties (due to the presence of active phenolic compounds) and androgenic activity. Ginger increases fertility and improves semen quality of sperm by disrupting the production of free radicals, plum-meting oxidative stress, dissolving oxidative chain reactions, and altering the levels of sex hormones (such as testosterone).

So, this research was conducted to examine the effect of ginger and black seed oils on the fertility of male rats **Gholami-Ahangaran *et al.*, (2021).**

## MATERIALS AND METHODS

### Materials

- Lead acetate was purchased from El-Gomhoria Company, Cairo, Egypt. The dose of lead acetate (60 mg/kg body weight) was used three times per week every other day orally in order to induce infertility **Offor et al., (2019).**
- Casein, all vitamins and minerals ingredients cellulose, and choline bitartrate were obtained from El-Gomhoria Company, Cairo, Egypt.
- Ginger and black seed oils were purchased from the Agricultural Research Center, Giza, Egypt.

### *Experimental animal:*

Thirty adult male albino rats weighing about (170 ± 10g BW.) were obtained from Helwan Animal House Colony of the Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Cairo, Egypt.

**Methods:**

***Chemical Analysis of black Seed and Ginger Oils:***

Chemical composition was determined according to **AOAC (2012)**, phenolic compounds were determined according to **Agilent Application Note, (2014)** While fatty acids content was determined according to **ISO, (2017)** at Food Technology Research Institute, Giza, Egypt.

***Preparation of Basal Diet:***

The basal diet was formulated according to the AIN-93M diet **Reeves *et al*., (1993).**

***Experimental Design:***

The experiment was conducted at Biological Studies Research Labs, Agricultural Research Center, Giza, Egypt. Thirty rats were housed in good conditions in standard cages at room temperature (25±3 °C) with a 12 h dark/light cycle. They were left for seven days as an adaptation period and they were allowed **to** feed standard laboratory diet and water. After the adaptation period, rats were divided into five groups, **Group 1:** The control negative was fed on a basal diet. **Group 2:** The control positive was fed on a basal diet and received lead acetate. **Group 3:** the same as group 2 with 5 % ginger oil replaced from soy oil in basal diet formula. **Group 4:** the same as group 2 with 5% black seed oil substituted with soy oil in basal diet formula. **Group 5:** as the same as group 2 with 2.5 % ginger and 2.5% black seed oils substituted with soy oil in basal diet formula.

***Biological Evaluation:***

Feed intake was recorded daily and animals were weighed at the beginning and twice a week throughout the experimental period. Body weight gain (BWG), feed intake (FI) as well as efficiency ratio (FER) were calculated according to the method of **Chapman *et al*., (1959)** and organ relative weight was estimated at the end of the experiment.

***Blood Collection and Serum Separation:***

At the end of the experimental period (8 weeks), rats were fasted overnight before sacrificing, and blood samples were collected from each rat and were centrifuged at 3000 rpm for

15 min to obtain the serum for biochemical analysis.

***Biochemical analysis:***

Serum total cholesterol (TC) was determined according to **Richmond, (1973).** Triglyceride (TG) was determined according to **Wahlefeld, (1974).** High-density Lipoprotein cholesterol (HDL-c) was determined according to **Warnick *et al.*, (1985).** Low-density Lipoprotein cholesterol (LDL-c) and very Low-density Lipoprotein cholesterol (VLDL-c) were estimated according to the equation described by **Friedewald *et al.*, (1972).** The Atherogenic index in plasma was calculated by using the formula described by **Nwagha *et al.*, (2010).** Serum Malondialdehyde (MDA) and catalase (CAT) enzymes were determined according to **Shin, (2009)** and **Góth, (1991),** respectively**.** Serum total testosterone level was determined according to **Wilke and Utley, (1987**).

***Epididymis sperm analysis***

Sperm samples were collected from the distal region of the epididymis (cauda) according to **Mali *et al.*, (2002).** Sperm samples were used for the evaluation of count, motility, and morphology according to **Narayana *et al.*, (2005).**

***Assessment Sperm Count***

The settled sperm were counted and evaluated per 250 small squares of a hemocytometer according to **Seed *et al.*, (1996)** and sperm count, motility, sperm life as well as sperm abnormalities were evaluated according to **Blom, (1983).**

***Histological examination***

Samples of histological examination were taken from the testes of rats in different groups and examined according to **Bancroft *et al.*, (1996).**

***Statistical Analysis:***

All obtained results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean± standard error (SE) according to **Armitage et al., (2002)**. All differences were considered significant if P-values were (P< 0.05).

## RESULT AND DISCUSSION

Table (1) shows the ginger and black seed oils' proximate chemical analysis. Data revealed that the saturated fatty acids in ginger oil were palmitic and stearic acids with values of 13.92 and 05.03 mg total phenolic content as Gallic acid equivalent (GAE)/g, respectively but in black seed oil, palmitic and stearic acids recorded 12.17 and 07.46 mg GAE/g, respectively. It means that ginger had the same value approximately of total saturated fatty acids compared to black seed oil recorded at 19.63 vs. 18.95 mg GAE/g, respectively.

The unsaturated oleic fatty acid in ginger and black seed oils was 41.48 vs 26.0 mg GAE/g, respectively as well as linoleic acid in ginger and black seed oils 38.90 vs 54.46 mg GAE/g, respectively. It means that black seeds had higher values of unsaturated fatty acids compared to ginger oil with total values 81.06 vs. 80.38 mg GAE/g, respectively.

**Maric *et al.*, (2020),** proved that ginger oil has higher values of unsaturated fatty acids like linoleic acid (c=18:2), and oleic acid (c=18:1) and higher than saturated fatty acids like stearic acid (c=18:0) and palmitic acid (c=16:0), which was in a line with these research results.

**Albakry *et al.,* (2022),** results are also in harmony with the above findings which reported that, the fatty acid composition of black seed. In this study, the levels of linoleic acid, oleic acid, palmitic acid, and stearic acid were high in the black seed oil so black cumin oil has a higher quantity of unsaturated fatty acids than other vegetable oils, indicating benefits to human health.

Table (2) shows the total phenolic content of both ginger and black seed oils. The ginger oil had the highest concentration of total phenolic content than black seed oil with mean values of 88.12 vs 65.57 mg GAE/g, respectively.
Food oils are thought to have radical scavenging properties due to their total phenolic content which has been linked to oil lipid oxidation **Karrar *et al.,* (2019).** Phenolic compounds are known to possess antioxidant in vitro capacity and shown to have a higher potential than vitamins C, E, and carotenoids. The phenolic consumption showed a positive correlation towards a reduced risk of various diseases related to oxidative stress. Ginger's pharmacological effect is mainly caused by 6-gingerol, 6-zogaol, and zingerone compounds besides phenolic compounds and another flavonoid. The pharmacological activity of ginger rhizome is due to a non-volatile compound of phenolic such as gingerol **Mahmudati *et al.,* (2020)**.

**Yancheshmeh *et al.,* (2022),** also reported that phenolic compounds can function as antioxidants due to their capacity to chelate metal ions and donate hydrogen atoms or electrons to stabilize free radicals. This prevents food products, particularly oils and fatty acids, from oxidizing.

Table (3) illustrates the effect of oils from ginger, black seeds, and their mixture on feed intake, body weight gains, and feed efficiency ratio of experimental male albino rats inducted with infertility. Mean feed intake values of the +ve control group decreased compared to the –ve control rat group with a mean value of 17.00 vs.19.00 g/d, respectively. Whereas, rats fed on 5% ginger, 5% black seed oil, 2.5% ginger, and 2.5% black seed oils mean values increased compared with +ve control rats with mean values of 17.5, 18.2, and 18.9 vs. 17.0 g/d, respectively.

Regarding the body weight gain, rats of the +ve control group significantly decreased compared to –ve control rats with mean values of 51.22 vs 62.8%, respectively. On the other hand, rats fed on 5% ginger, 5% black seed oil, 2.5% ginger, and 2.5% black seed oils mean values were significantly increased compared to the +ve control group with mean values of 45.4, 41.8, and 52.8 vs. 51.22 %, respectively.

Furthermore, the feed efficiency ratio (FER) data indicated that the +ve control group was significantly decreased compared with the –ve control group with mean values of 0.26 vs 0.32, respectively. Therefore, rats of groups 3 - 5 which were fed on 5% ginger, 5% black seed, and 2.5% ginger with 2.5% black seed oils were significantly increased compared to +ve control group rats with mean values 0.32, 0.28, and 0.33 vs 0.26, respectively.

**Ismail *et al.*, (2021),** findings were by the above results reported that ginger and black seed oil are known to have bioactive compounds (e.g., gingerol, thymoquinone) that can affect appetite regulation. Studies have shown that these oils might reduce feed intake due to their bitter compounds or effects on satiety hormones.

Rats fed ginger extract or oil showed a decrease in feed intake compared to the +ve control group. The reduction was attributed to the presence of bioactive compounds such as gingerol and shogaol, which can influence appetite-regulating hormones. Moreover, studies indicated that ginger enhances metabolism, improves lipid profiles, and can reduce fat accumulation, often leading to reduced weight gain.

Similarly, black seed oil has been found to modulate lipid metabolism and reduce weight gain in animal studies. Bioactive compounds in this oil can increase energy expenditure and reduce fat synthesis, making the conversion of feed into body weight less efficient. Therefore, research supports the finding that treatments with functional oils often lead to improved FER in experimental animals. Thymo-quinone, in black seed oil which is the active compound, promotes energy expenditure while supp-ressing fat deposition.

Table (4) shows the effect of ginger and black seed oils on total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), very low-density lipoprotein cholesterol (VLDL-c), and low-density lipoprotein cholesterol (LDL-c) on the fertility of male rats.

Regarding total cholesterol (TC), rats of the +ve control group which were treated with lead acetate significantly increased compared to rats of the -ve control group with mean values of 131.06 vs 83.72 mg/dl, respectively. Rats of all tasted groups (G 3-5) which were fed 5% ginger oil, 5% black seed oil, and 5 % oils mixture (1:1), respectively significantly decreased compared to the +ve control group with mean values 122.17, 113.24, 95.83 vs. 131.06 mg/dl. The best improvement among all treated groups was recorded in group 5 which was fed 2.5% ginger and 2.5% black seed oils.

Data revealed that triglycerides (TG) of rats in the +ve control group which was treated with lead acetate, significantly increased compared to rats of the -ve control group with mean values 305.20 vs 163.35 mg/dl, respectively. Whereas, rats were fed 5% ginger oil, 5% black seed oil and 2.5 ginger with 2.5 black seed oils mixture significantly decreased compared with the +ve control group with mean values 278.80, 248.60, 190.90 and 305.20 mg/dl, respectively. The best improve-ment among all treated groups was recorded in group (5) which was fed 2.5 ginger and 2.5 black seed oils.

Regarding high-density lipoprotein cholesterol (HDL-c), rats of the +ve control group which were treated with lead acetate, significantly decreased compared with rats of the -ve control group with mean values of 19.23 vs 30.32 mg/dl, respectively.

Rats of all tasted groups which were fed 5% ginger oil, 5% black seed oil, and 2.5 ginger with 2.5 black seed oils mixture significantly increased compared with the +ve control group with mean values 22.75, 25.64, 27.11, and 19.23 mg/dl, respectively. The highest improvement among all treated groups was recorded in rats of group three which was fed 5% ginger oil.

Data presented in the same Table showed the mean values of very low-density lipoprotein cholesterol (VLDL-c). Rats of the +ve control group which were treated with lead acetate significantly increased compared with rats of the -ve control group with mean values of 61.04 vs 32.67 mg/dl, respectively.

Rats of all tasted groups which were fed 5% ginger oil, 5% black seed oil and 2.5 and 2.5 of the oils mixture significantly decreased compared with the +ve control group with mean values 55.76, 49.72, 38.18, and 61.04mg/dl, respectively. The best improvement among all treated groups was recorded in group (5) which was fed 2.5 ginger with 2.5 black seed oils.

Results of low-density lipoprotein cholesterol (LDL-c) that were shown in the same Table revealed that rats of the +ve control group which were treated with lead acetate significantly increased compared with rats of the -ve control group with mean values 50.79 vs 20.73 mg/dl, respectively.

On the other hand, rats of all tasted groups which were fed 5% ginger oil, 5% black seed oil, and 2.5 and 2.5 of the oils mixture significantly decreased compared with the +ve control group with mean values 43.66, 37.88, 30.54, and 50.79 mg/dl, respectively. The best improvement among all treated groups was recorded in group (5) which was fed 2.5 ginger and 2.5 black seed oils.

The results of the Atherogenic index are shown in the same Table. They revealed that rats of the +ve control group treated with lead acetate significantly increased compared with rats of the -ve control group, with mean values of 6.81 vs 2.76 mg/dl, respectively.

On the other hand, rats of all tasted groups which were fed 5% ginger oil, 5% black seed oil and 2.5 and 2.5 of the oils mixture significantly decreased compared with the +ve control group with mean values 3.53, 4.41, 5.37, and 6.81 mg/dl, respectively. The best improvement among all treated groups was recorded in group three which was fed 5% ginger oil.

The research results were in agreement with **Ghosh *et al.*, *(*2014*)*,** who reported that there was an increase in the level of total cholesterol, triglyceride, VLDL, and LDL cholesterol in lead acetate-treated rats which was in line with the fact that exposure to lead acetate causes risky altered lipid profile. The lead acetate exposure increases the synthesis of cholesterol and elevates total cholesterol, triglyceride, and LDL cholesterol as well as the Total cholesterol: HDL cholesterol and LDL: HDL ratios which led to increases in the risk of cardiovascular diseases.

Recent clinical trials with ginger supplementation reported that ginger supplementation resulted in a remarkable reduction in low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglyceride (TG) levels, as well as an increase in high-density lipoprotein cholesterol (HDL-C) concentration **Pourmasoumi *et al.*, (2018).**

Ginger affects adipocyte remodeling so its supplementation effectively reduces HF-mediated adipocyte hypertrophy with elevated lipogenic levels **Ahmed *et al.*, (2023).**

**Cicero *et al.*, (2017),** reported that modern clinical studies revealed that black cumin oil exerts multiple pharmacological properties such as hypolipidemic (low-density lipoprotein, triglyceride, and total cholesterol concentration lowering) *N. sativa* is rich in polyunsaturated fatty acids (PUFA), which contributors to the inhibition of VLDL secretion, and hormone-sensitive lipase, destruction of apo-B100, increasing clearance of triglycerides using enhancing endothelial lipoprotein lipase activity and increasing fatty acid oxidation. Flavonoids and thymoquinone (TQ) in N. sativa may result in decreasing cholesterol synthesis via suppressing 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-COAR) gene expression, upregulating LDL-receptors in the liver and finally increasing uptake of LDL from blood circulation. Natural nigella in N. *sativa* similar to synthetic clofibrate leads to reduced serum triglyceride **Hallajzadeh *et al.*, (2020**).

The research results were in agreement with **Ghosh *et al.*,**

***(*2014*)*,** who also observed an increase in the ratios of total cholesterol: HDL cholesterol and LDL: HDL in the rats treated with lead acetate intraperitoneally, The current finding is in agreement with the fact that exposure to lead acetate causes altered lipid profile, increased total cholesterol and decreased HDL cholesterol and that lead acetate exposure increases synthesis of cholesterol and transport to peripheral tissues whereas reverse cholesterol transport to the liver is not affected. The present study saw a significant decrease in the level of HDL cholesterol in the serum of the rats treated sub-chronically with lead acetate which was observed to be protected and maintained at normal levels on pre-treating the animals.

**Asghari-Jafarabadi and Khalili (2022)** determine ginger's optimum dose and improve blood lipid levels. Ginger's active components responsible for its effects are mainly gingerols and their related dehydration products and volatile oils. Ginger decreased the Atherogenic index of plasma compared with the +ve control group.

**Li *et al*., (2020),** results showed that black seed oil was more beneficial to the recovery of hyperlipidemia and fatty liver, the chemical composition of black cumin seeds is limited, volatile compounds, fatty acid profile, and bioactive properties of oil extracted from black cumin seeds, the minerals in black seeds (sodium, iron, zinc, and copper) are relatively high. The amount of black seed consumed will not predict the nutritional status of these minerals.

Data presented in Table (5) showed the testosterone of male rats treated with ginger and black seed oils. The rats of the +ve control group were treated with lead acetate, which significantly decreased compared to rats of the -ve control group with mean values of 0.68 vs 2.38 ng/ml, respectively. The testosterone of rats in all tasted groups which were fed 5% ginger oil, 5% black seed oil and 2.5 and 2.5 of the oils mixture significantly increased compared with the +ve control group with mean values 1.91, 1.66, 1.88, and 0.68 ng/ml, respectively. The best improv-ement among all treated group was regarded in group (3) which was fed 5% ginger oil.

The obtained results were in agreement with **Oyeyemi *et al*., (2022),** who reported that sperm viability was significantly reduced in the +ve control group treated with lead acetate compared with the –ve control group. The percentage of abnormal sperm morphology was significantly decreased in both oils treated groups compared with the +ve control group.

Moreover, **Hosseini *et al*., (2016**), indicated that ginger improves sperm function and semen quality which raises the rate of fertilization. Spermatogenesis and steroidogenesis are the processes in which sperm and androgens primarily are produced according to the findings of this study. The rate of DNA damage in infertile individuals treated with a ginger extract was much lower than in the control group.

**Chandeliya and Singh, (2023),** also reported that ginger contains a high concentration of minerals that help increase testosterone levels, such as manganese. **Leisegang *et al*., (2021),** also proved that black seed oil improved body weight, sperm concentration, and serum testosterone levels with no significant effect on progressive and non-progressive motility. The results of this study in the context of the current literature suggest that black seed may be beneficial in male reproduction, the mechanisms include a reduction of oxidative stress through increased endogenous antioxidant regulation and inflammation.

Table (6) presents the levels of catalase (CAT) and malondialdehyde (MDA) of infertility rats treated with ginger and black seed oils and regarding CAT, rats of the +ve control group, which were treated with lead acetate, significantly decreased compared with rats of the -ve control group, with mean values of 20.16 vs. 33.28 µ/l, respectively.

Rats of all tasted groups which were fed 5% ginger oil, 5% black seed oil and 2.5 and 2.5 of the oils mixture significantly increased compared with the +ve control group with mean values 32.56, 30.09, 30.43, and 20.16 µ/l, respectively. Group 4 which was treated with 5% black seed oil did not significantly differ compared with the other treated group that received the mixture oils. Whereas, the best improvement among all treated group was recorded in group 3 which was fed 5% ginger oil.

With regard to malondialdehyde (MDA), rats in the +ve control group which was treated with lead acetate, significantly decreased compared to rats of the -ve control group with mean values 1.77 vs 1.20 nmol/ml, respectively.

Rats of all treated groups which were fed 5% ginger oil, 5% black seed oil and 2.5 and 2.5 of the oils mixture significantly decreased compared to +ve control group with mean values 1.24, 1.45, 1.43 vs 1.77 µmol/ml, respectively. Group 4 which was treated with 5% black seed oil did not significantly differ compared with group 5 treated with 2.5 with 2.5 oils mixture. The best improvement among all treated group was recorded in group 4 which was fed 5% black seed oil followed by rats of group 5 treated with mixed oils.

**Kandeil *et al.*, (2020),** reported that the administration of lead acetate has a bad effect on testes by causing a decline in sperm count and viability. It also decreases the antioxidant levels in testicular tissue as glutathione, superoxide dismutase and catalase, and results in increased lipid peroxidation in this study in harmony which was by these research findings.

**Cayir *et al.*, (2011),** reported that when feeding ginger oil, there are endogenous antioxidant defense mechanisms, including antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase, in protecting or reducing oxidative stress.

**Latif *et al.*, (2021),** that black seed oil maintains the lowest level of reactive oxygen species (ROS) in cells in Enzymatic and non-enzymatic antioxidants such as CAT, GST, GPx, and GSH. The activities of antioxidant enzymes differ among different tissues and were found to be higher in those tissues that have higher oxidative properties so, this study is in a harmony with above research results.

**Oyeyemi *et al*., (2020)**, proved that lead acetate increased testicular MDA concentration in the positive group and this could be due to the accumulation of lead in the testes as observed in this study. The increase in MDA and a reduction in testicular catalase activities were observed in lead-treated animals which was in line with previous results of this study. Moreover, **Morvaridzadeh *et al.*, (2021),** reported that ginger consumption significantly reduced plasma MDA concentration and significantly increased GPx activity. In addition, the results indicated that ginger supplementation increases CAT activity non-significantly. This is not the first systematic review and meta-analysis investigating the impact of supplemental ginger on OS parameters in human adults.

**Stoilova *et al.*, (2007),** reported that several possible mechanisms be attributed to the effect of ginger on lipid peroxidation. The administration of ginger has been shown to significantly reduce thiobarbituric acid-reactive substance levels, which can be considered to be an indicator of oxidative stress damage and lipid peroxidation.

**Yousefi *et al.*, (2021),** reported that the black seed significantly reduced lipid peroxidation levels and measured as malondialdehyde. This study showed that black seeds displayed significantly lower levels of MDA. Antioxidant enzymes such as CAT, GPx, and SOD have protective effects by preventing uncontrolled generation of ROS. Therefore, the inclusion of black seed activated some antioxidant enzymes (SOD and GPx), which was related to the lower oxidative stress levels (MDA) observed in the treated group which is in harmony with the research results.

Table (7) illustrates that regarding the sperm concentration, the +ve control group was significantly decreased compared to the –ve control group with mean values of 29.50 vs 84 $x10^6$/ml, respectively (Table 8). Therefore, Rats of all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture, significantly increased compared to the +ve control group with mean values 43.50, 60.40, 50.33 vs. 29.50 $x10^6$/ml, respectively. The best improvement among all treated group was recorded in group 4 which was fed 5% black seed oil.

The percentage of all live sperm of rats in the +ve control group was significantly decreased compared to the –ve control group with mean values of 49.83 vs 72.83%, respectively. Whereas, all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture were significantly increased compared to the +ve control group with mean values 59.6, 63.8, 62.8 vs. 49.83%, respectively. The best improvement among all treated groups was recorded in group 4 which was fed 5% black seed oil.

Regarding sperm abnormalities, the +ve control group was significantly increased compared to the –ve control group with mean values of 38.00 vs 18.00%, respectively. Rats of all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture were significantly decreased compared to the +ve control group with mean values of 24.00, 19.50, 21.5 vs. 38.00%, respectively. The best improvement among all treated groups was recorded in group 4 which was fed 5% black seed oil.

Sperm without the head hook in the +ve control group was significantly increased compared to the –ve control group with mean values of 6.00 vs 0.66%. Therefore, rats of all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture significantly decreased compared to the +ve control group with mean values 5.50, 2.66, 3.60 vs. 6.00%, respectively. The best findings among all treated groups were recorded in group 4 which was fed 5% black seed oil.

About banana shape head, the +ve control group was significantly increased compared to –ve control group with mean values 4.33 vs 0.10% respectively but rats of all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture significantly increased compared to the +ve control group with mean values 2.40, 0.80, 1.16 vs. 4.33%, respectively. The best improvement among all treated groups was recorded in group 4 which was fed 5% black seed oil.

Concerning the amorphous shape head, the +ve control group was significantly increased compared to the –ve control group with mean values of 6.16 vs 1.50%. Rats of all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture were significantly decreased compared to the +ve control group with mean values of 5.40, 4.16, 4.99 vs. 6.16%, respectively. The best results among all treated groups were recorded in group 4 which was fed 5% black seed oil.

Data presented in the same Table showed that the sperm abnormal tail of the +ve control group was significantly increased compared to the –ve control group with mean values of 20.60 vs 2.50%. Rats of all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture significantly decreased compared to the +ve control group with mean values 13.16, 5.66, 11.50 vs. 20.60%, respectively. The best improvement among all treated groups was recorded in group 4 which was fed 5% black seed oil.

Concerning sperm motility, the +ve control group was significantly increased compared to the –ve control group with mean values of 27.83 vs 47.50%, respectively. Rats of all tasted groups which were fed 5% ginger, 5% black seed oils and 2.5 and 2.5% oils mixture significantly increased compared to the +ve control group with mean values 38.33, 42.50, 41.00 vs. 27.83%, respectively. The best improvement among all treated groups was recorded in group 4 which was fed 5% black seed oil.

**Ileriturk *et al.*, (2021),** findings were following these obtained results which proved that the exposure to lead acetate decreased sperm concentration count and sperm motility while it increased the abnormal sperm count. Comparable impaired sperm parameters were observed in rats exposed to lead acetate. Feeding lead acetate led to the enhancement of oxidative stress and a reduction in testicular antioxidants, toxic effects on spermatocytes, and changes in the hypothalamic-pituitary-gonadal (HPG).

**Herve *et al.*, (2018),** showed that ginger might enhance the fertility of males, which was an increased number of live sperm, total antioxidant capacity of the seminal plasma, sperm membrane integrity, forward motility, and sperm penetration.

**Ali, (2019),** reported that the mechanisms of Ginger increased sperm production to improve testes growth by enhancing the development of the seminiferous tubules and germ cells and semen quality by suppressing the oxidative damage induced in the testes via the activation of antioxidant enzymes, such as superoxide dismutase and catalase. There were increases in testes weight, ejaculated volume, sperm concentration, and motility but decreases in dead sperm and abnormal sperm. These findings showed that *Zingiber officinale* enhances spermatogenesis.

**Leisegang *et al.*, (2021),** Reported that the black seed oil was similarly effective in reducing total body weight and increasing sperm concentration and serum testosterone levels in rats. Progressive motility and viability were not affected by black seed oil. Black seed oil may offer benefits in male immune regulation, and antioxidant activity. The mechanisms include a reduction of oxidative stress through increased endogenous antioxidant regulation, and immune regulation through a reduction in inflammatory cytokines.

All treated groups from 3-5 showed some histological improvements when compared to the +ve control group which was in harmony with all the above biochemical assays.

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**Table (1): Fatty acids content of ginger and black seed oils**

| Fatty acids (mg GAE/g) | | Ginger (%) | Black seed (%) |
|---|---|---|---|
| Saturated Fat | Palmitic Acid (c16:0) | 12.17 | 13.92 |
| | Stearic Acid (c18:0) | 07.46 | 05.03 |
| | Total | 19.63 | 18.95 |
| Unsaturated Fat | Oleic Acid (c18:1) | 41.48 | 26.60 |
| | Linoleic Acid (c18:2) | 38.90 | 54.46 |
| | Total | 80.38 | 81.06 |

**Table (2): Total Phenolic content of ginger and black seed oils**

| Phenolic content | mg GAE/g |
|---|---|
| Ginger Oil | 88.12 |
| Black Seed Oil | 65.57 |

**Table (3): Effect of ginger and black seed oils on feed intake (FI), body weight gain (BWG), and feed efficiency ratio (FER) of male rats**

| Groups | FI (g/d/rat) | BWG (%) | FER |
|---|---|---|---|
| G1: -ve Control | 19 | 62.8±2.01[a] | 0.32±0.001[a] |
| G2: +ve Control | 17 | 51.22±1.90[c] | 0.26±0.003[c] |
| G3: 5% ginger oil | 17.5 | 45.4±1.77[d] | 0.32±0.001[a] |
| G4: 5% black seed oil | 18.2 | 41.8±1.02[d] | 0.28±0.002[b] |
| G5: 2.5 + 2.5% ginger and black seed oils | 18.9 | 52.82±1.85[b] | 0.33±0.002[a] |

******Mean values are expressed as means ± SE.*

***Mean values at the same column with the same superscript letters are not statistical. significant at P<0.05.***

**Table (4): The effect of ginger and black seed oils on the lipid profile of male rats inducted with infertility**

| Groups | G1: -ve Control | G2: +ve Control | G3: 5% GO | G4: 5% BSO | G5:2.5% GO + 2.5% BSO |
|---|---|---|---|---|---|
| TC | 83.72 ± 4.15[e] | 131.06± 6.12[a] | 122.17± 5.09[b] | 113.24± 3.32[c] | 95.83± 2.99[d] |
| TG | 163.35± 2.87[e] | 305.20± 4.11[a] | 278.80± 3.71[b] | 248.60± 2.99[c] | 190.90± 3.08[d] |
| HDL | 30.32 ± 1.09[a] | 19.23 ± 2.11[e] | 22.75 ± 1.00[d] | 25.64 ± 1.34[c] | 27.11± 0.89[b] |
| VLDL | 32.67 ± 0.96[e] | 61.04 ± 0.11[a] | 55.76 ± 0.28[b] | 49.72 ± 0.66[c] | 38.18 ±0.10[d] |
| LDL | 20.73 ± 0.20[e] | 50.79 ± 0.11[a] | 43.66 ± 0.41[b] | 37.88 ± 0.33[c] | 30.54± 0.51[d] |
| TC/HDL | 2.76 ± 0.09[e] | 6.81± 0.53[a] | 5.37± 0.46[b] | 4.41± 0.28[c] | 3.53± 0.70[d] |
| LDL/HDL | 0.68±0.003[e] | 2.64± 0.17[a] | 1.91±0.002[b] | 1.47±0.001[c] | 1.12±0.001[d] |

***Mean values are expressed as means ± SE.**

***Mean values at the same raw with the same superscript letters are not statistical. significant at P<0.05. GO= ginger oil BSO= black seed oils**

**Table (5): The effect of ginger and black seed oils on the testosterone hormone of male rats inducted with infertility**

| Groups | Testosterone (ng/ml) |
|---|---|
| G1: -ve Control | 2.38±0.01[a] |
| G2: +ve Control | 0.68±0.002[d] |
| G3: 5% GO | 1.91±0.008[b] |
| G4: 5% BSO | 1.66±0.012[c] |
| G5:2.5% GO + 2.5% BSO | 1.88±0.019[b] |

****Mean values are expressed as means ± SE.***
***Mean values at the same column with the same superscript letters are not statistical. significant at P<0.05. GO= ginger oil BSO= black seed oils***

**Table (6): The effect of ginger and black seed oils on catalase (CAT) and malondialdehyde (MDA) of male rats inducted with infertility**

| Groups | CAT (µ/l) | MDA (nmol/ml) |
|---|---|---|
| G1: -ve Control | 33.28±0.09[a] | 1.20±1.06[c] |
| G2: +ve Control | 20.16±1.09[c] | 1.77±1.23[a] |
| G3: 5% GO | 32.56±1.40[a] | 1.24±0.99[c] |
| G4: 5% BSO | 30.09±1.32[b] | 1.45±1.18[b] |
| G5:2.5% GO + 2.5% BSO | 30.43±1.34[b] | 1.43±1.88[b] |

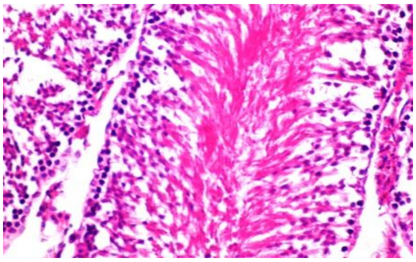
***Mean values are expressed as means ± SE.***
***Mean values at the same column with the same superscript letters are not Statistically. significant at P<0.05.*** **GO= ginger oil BSO= black seed oils**

**Table (7): The effect of ginger and black seed oils on the semen of male rats inducted with infertility**

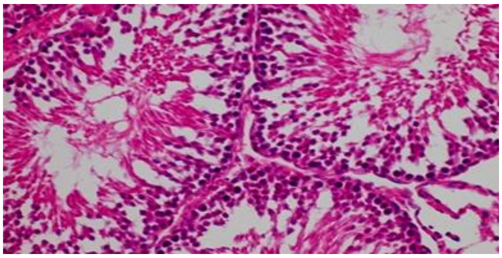
| Groups | Sperm concentration ($x10^6$/ml) | Percentage of live sperm (%) | Sperms abnormalities (%) | Without hook head (%) |
|---|---|---|---|---|
| G1: -ve Control | $84.00±1.67^a$ | $72.83±2.73^a$ | $18.00±1.60^e$ | $0.66±0.09^d$ |
| G2: +ve Control | $29.50±1.62^e$ | $49.83±1.53^d$ | $38.00±1.63^a$ | $6.00±0.87^a$ |
| G3: 5% GO | $43.50±1.90^d$ | $59.60±0.37^c$ | $24.00±1.98^b$ | $5.50±0.04^a$ |
| G4: 5% BSO | $60.40±1.46^b$ | $63.83±1.29^b$ | $19.50±1.04^d$ | $2.66±0.18^c$ |
| G5: 2.5% GO + 2.5% BSO | $50.33±0.71^c$ | $62.25±1.32^b$ | $21.50±1.86^c$ | $3.60±0.04^b$ |
| **Groups** | **Banana shape head (%)** | **Amorphous shape head (%)** | **Abnormal Tail (%)** | **Sperm Motility (%)** |
| G1: -ve Control | $0.10±0.00^e$ | $1.50±0.56^e$ | $2.50±0.17^e$ | $47.50±1.61^a$ |
| G2: +ve Control | $4.33±0.37^a$ | $6.16±0.35^a$ | $20.60±1.80^a$ | $27.83±1.06^d$ |
| G3: 5% GO | $2.40±0.24^b$ | $5.40±0.77^b$ | $13.16±1.09^b$ | $38.33±0.80^c$ |
| G4: 5% BSO | $0.80±0.00^d$ | $4.16±0.87^d$ | $5.66±0.88^d$ | $42.50±1.02^b$ |
| G5: 2.5% GO + 2.5% BSO | $1.16±0.40^c$ | $4.99±0.34^c$ | $11.50±0.98^c$ | $41.00±1.09^b$ |

***Mean values are expressed as means ± SE.**
***Mean values at the same column with the same superscript letters are not statistical. significant at P<0.05.** **GO= ginger oil BSO= black seed oils**

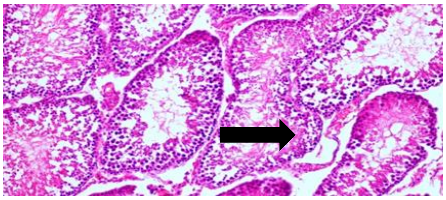
## Photo of Testes Histopathology



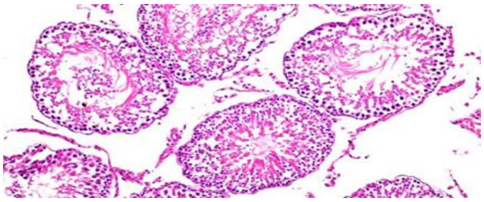
**Test rats' group (1) shows the normal histological structure of seminiferous tubules with normal spermatogenic cells (H&E X 400).**



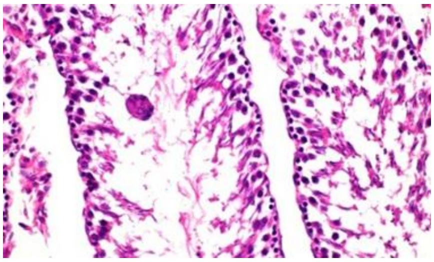
**Testes rats' group (2): revealing irregular contouring of basal lamina of seminiferous tubules, reduction in the number of germ cells with the appearance of vacuoles or spaces in the germinal epithelium of seminiferous tubules (H&E X 400).**



**Test rats' group (3), showed irregular contouring of the basal lamina of seminiferous tubules, dilatation, and congestion of testicular blood vessels (arrow) in addition to necrobiotic changes with depletion of the germ cells (H&E X 200).**



**Testes rats' group (4), some seminiferous tubules showed desquamation of the germ cells in their lumen others showed a reduction in the number of germ cells with the appearance of spaces in the germinal epithelium in addition to interstitial edema, dispersion, and irregular contouring of the basement membrane of the seminiferous tubules (H&E X 200).**



**Testes frats' group (5), shows multinucleated giant cells in the lumen of seminiferous tubules with a reduction in the number of germ cells (H&E X 400)**

# تأثير زيوت الزنجبيل والحبة السوداء على خصوبة ذكور الجرذان

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## المستخلص العربي

*أجريت هذه الدراسة للتحقيق في التأثيرات الحيوية لزيوت الزنجبيل وحبة البركة على خصوبة ذكور الفئران. تم توزيع ثلاثين جرذاً سلالة سبراج داولي بشكل عشوائي بعد فترة التكيف إلى مجموعتين رئيسيتين، المجموعة الرئيسية الأولى (6 جرذان) تم إبقاؤها على النظام الغذائي الأساسي كمجموعة ضابطة سلبية. تم إعطاء بقية الحيوانات (ن = 24) خلات الرصاص (60 مجم / كجم وزن الجسم) عن طريق الفم عن طريق التغذية الأنبوبية ثلاث مرات في الأسبوع من أجل تحفيز العقم. بعد ذلك، تم تقسيم الجرذان المصابة بالعقم إلى 4 مجموعات فرعية. تم تغذية المجموعة الفرعية الأولى على النظام الغذائي الأساسي لمدة 8 أسابيع وعملت كمجموعة ضابطة إيجابية . تم تغذية المجموعات الفرعية الأخرى، مثل المجموعة الفرعية 2، على نظام غذائي أساسي يحتوي على 5٪ زيت زنجبيل و 5٪ زيوت حبة البركة و 2.5٪ وتم تقدير محتوى الأحماض الدهنية ومحتوى الفينولي الكلي لكلا الزيتين. في نهاية الفترة التجريبية (8 أسابيع)، تم حساب تناول الغذاء وزيادة وزن الجسم ونسبة كفاءة التغذية. تم جمع الأعضاء (الخلايا المنوية) للفحص النسيجي. تم جمع عينات الدم لتقدير صورة الدهون والتستيستيرون و CAT و MD . أشارت النتائج إلى تحسن صورة الدهون بشكل ملحوظ في جميع المجموعات المختبرة مقارنة بالمجموعة الضابطة الإيجابية. علاوة على ذلك، تحسنت إنزيمات مضادات الأكسدة SOD و CAT بشكل ملحوظ في جميع المجموعات المختبرة مقارنة بالمجموعة الضابطة الإيجابية. ومع ذلك، انخفض MDA في المصل بشكل ملحوظ في المجموعات 3 و4 و5 على التوالي عند مقارنتها بالمجموعة الضابطة الإيجابية. يمكن أن نستنتج أن زيوت الزنجبيل وحبة البركة لها تأثير محتمل على الخصوبة وبالتالي توصي هذه الدراسة بزيادة بتناول زيت الزنجبيل وحبة البركة في النظام الغذائي يمكن أن يكون مفيدًا للمرضى الذين يعانون من انخفاض الخصوبة.*

**الكلمات المفتاحية:** *زيت الزنجبيل - زيت حبة البركة - الخصوبة – الجرذان*