Nigella sativa Oil Improves Follicular Reserve in Cyclophosphamide-Induced Ovarian Toxicity: A Histomorphological and Hormonal Assessment in Female Rats

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Posted Date: 4 October 2024

doi: 10.20944/preprints202408.1105.v2

Keywords: Nigella sativa; Reproduction; Histomorphology; Ovaries; toxicity

Article

Nigella sativa Oil Improves Follicular Reserve in Cyclophosphamide-Induced Ovarian Toxicity: A Histomorphological and Hormonal Assessment in Female Rats

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Abstract: Histomorphological studies were conducted to evaluate the effects of NS-Oil (NS) on ovarian toxicity induced by cyclophosphamide in female rats. The research aimed to assess the histological changes in ovarian tissue and correlate these findings with alterations in serum gonadotropin levels. The experimental design involved three (3) experimental groups treated with cyclophosphamide, NS-Oil oil, or a combination, with control group for comparison. Histological assessments of ovarian sections were performed to analyze structural integrity, follicular development, and toxicity signs, while serum samples were analyzed for estradiol, progestin levels. Cyclophosphamide administration significantly disrupted ovarian architecture, reducing follicle count and causing follicular degeneration. Conversely, NS-Oil oil treatment exhibited protective effects, restoring normal histological features and improving gonadotropin levels. Image J software, calibrated with a table micrometer, was used to measure diameters of follicles, follicular cells, and oocytes. Primary, secondary, and tertiary follicles were classified according to Oktay et al. (2017). Body weight showed no significant difference across groups, but ovarian weight decreased in cyclophosphamide-treated groups compared to controls ($p < 0.05$). However, ovarian weight increased dose-dependently in NS-Oil-treated groups ($p < 0.05$). Cyclophosphamide reduced ovarian cortex and medulla volumes ($p < 0.05$), while NS-Oil treatment increased these volumes ($p < 0.05$). Pre-antral and antral follicle counts decreased with cyclophosphamide but increased following NS-Oil treatment ($p < 0.05$). Atretic follicle counts decreased in both cyclophosphamide and NS-Oil-treated groups ($p < 0.05$). The diameters of pre-antral and antral follicles, as well as follicular cells, decreased with cyclophosphamide but increased post NS-Oil treatment ($p < 0.05$). These results suggest that NS-Oil may offer a therapeutic strategy to mitigate ovarian damage associated with cyclophosphamide treatment, highlighting its potential role in preserving reproductive health in patients undergoing chemotherapy. Further investigations are warranted to elucidate the underlying mechanisms of action and the efficacy of NS-Oil in clinical settings.

Keywords: Nigella sativa; reproduction; histomorphology; ovaries

I. Introduction

A new study according to the WHO 2023, stated that large numbers of people are affected by infertility in their lifetime, making around 17.5% of the adult population. This is roughly estimated to be 1 in 6 couples worldwide experience infertility, highlighting the burden of infertility, and the need to sought for more studies to provide alternative approaches that is accessible and affordable fertility care for those in need (WHO 2023).

 doi:10.20944/preprints202408.1105.v2

Infertility, defined as the failure to conceive a recognized pregnancy after 12 months of unprotected intercourse, carries significant personal, societal and financial consequences (WHO, 2023). Infertility remains a highly prevalent condition worldwide, occurring in 8-12% of couples (Marcia et. al., 2015). However, the incidence varies from one region of the world to the other, being highest in the so-called infertility belt of Africa that including south Asia, sub-Saharan Africa, the Middle East and North Africa, Central and Eastern Europe and Central Asia (Mascarenhas et al., 2012). In contrast to an average prevalence rate of 10-15% in the developed countries (Alvarez, 2006), the prevalence of infertility has been notably highly variable in sub-Saharan Africa ranging from 20-46%. This has been attributed to high rate of sexually transmitted diseases, complications of unsafe abortions, and puerperal pelvic infections (Idrisa, 2005). About 30% of infertility is due to female problems, 30% to male problems, and 30% to combined male/female problems, while in 10%, there is no recognizable cause (Inhorn, 2015).

Nigella sativa, commonly known as black cumin, is an annual flowering plant belonging to the Ranunculaceae family. It is native to the Mediterranean Basin but has naturalized in various regions, including Southern Europe, Southeast Asia, Northern Africa, Eastern Africa, and North America (The Angiosperm Phylogeny Group et al. 2016). The plant is characterized by a slender stem (20-30 cm), linear leaves, delicate white or purplish flowers with 5-10 petals, and large capsules containing numerous small, sharp black seeds (Mozaffarian 2016). These seeds have been extensively studied for their phytochemical properties, which include essential oils (Burits and Bucar 2000), alkaloids (The Angiosperm Phylogeny Group et al. 2016), fatty acids (Nergiz and Otles 1993), sterols (Salama 1973), saponins (Taşkin et al. 2005), tannins (Nergiz and Otles 1993), flavonoids (Wray et al. 1997), natural organic acids (Bourgou et al. 2008), vitamins and minerals (Nergiz and Otles 1993).

Nigella sativa has a long history of traditional medicinal use in Asia for treating various ailments, such as headaches, infertility, fever, migraines, diabetes, hypertension, inflammation, and cancer (The Angiosperm Phylogeny Group et al. 2016; Mozaffarian 2016). The seeds are known for their antimicrobial, analgesic, diuretic, hepatoprotective, cardioprotective, and neuroprotective properties. From the pharmacological stand point, the whole plant is employed in Asia to treat several diseases including headache, infertility, fever, migraine, diabetes, hypertension, inflammations and cancer (Gholamnezhad et al. 2016). Indeed, the seeds are used in Asia to exert many properties including the antimicrobial, analgesic, diuretic, antioxytocic, antinociceptive, hepatoprotective, cardioprotective and neuroprotective ones (Gholamnezhad et al. 2016).

Fertility study of *Nigella sativa* seeds has shown to increase the weight of reproductive organs, sperm motility and count in cauda epidydimides and testicular ducts in the male rats, while there was an increase in the number of female pregnant rats (Al-Savaii *et al*, 2009). *Nigella sativa* oil increased the secretion of sexual hormones, which improved hepatic enzyme protein synthesis, white blood cell count, and blood cholesterol concentration (Farooq T. et. al., 2011).

Problem Statement/Justification

Despite advancements in healthcare services, medical interventions, and assisted reproductive technologies in gametes and embryo manipulations, such as Invitro fertilization (IVF), Infertility still remains a major problem that affects most couples all over the world. In Africa, impaired fertility is a well-known public health issue (WHO 2020). It is a common source of marital problem and disharmony, and is more aggravated in the African society. Several studies indicate that infertility is the most frequent reason for gynecological consultation in Nigeria (Okonofua, 2003). More than 50% of gynecological caseloads are as a result of infertility (Otuba, 2017), and over 80% of laparoscopic investigations are as a result of infertility (Okoye, 2020). The International Family planning perspectives in 2017 declared that about 33% of women aged 20-44years suffer from one form of infertility in Nigeria. A substantial proportion of all those affected in developing countries, live in extreme poverty and cannot afford established medical institutions, equipped to address cases of infertility. They resort to the use of traditional folklore medicine, which employs the use of plants and its phytochemical compounds, and is readily available, accessible and very much affordable. This study was aimed at investigating the cyclical changes and histomorphological effects of *Nigella sativa*

 doi:10.20944/preprints202408.1105.v2

oil on the ovaries, reproductive tract, hypothalamo-pituitary-ovarian axis, plasma gonadotropins and folliculogenesis in adult female wistar rats.

2. Methods

Experimental Design:

The research protocols were approved by the Institutional Animal Care and Use (IACU) protocol, University of Jos, issued by the ethical committee animal experimental unit with approval number: UJ/FPS/F17-00379.

Experimental Animals:

Twenty-four adults female Wistar rats weighing 120-140g were obtained from the Livestock section of the School of Veterinary and Medical Laboratory Sciences at the National Veterinary Research Institute (NVRI) in Vom. All experiments were conducted in the Vivarium of the same institution. The rats were housed in plastic cages under standard laboratory conditions, including room temperature, humidity, and a 12-hour light/dark cycle. The animals' cages were cleaned daily, and they were fed a standard laboratory diet (Grand Cereal Animal Feeds, Vom) and provided drinking water ad libitum. The rats were acclimated to this environment for two weeks before the study began. All animal care and procedures complied with the guidelines of the institute's ethical committee for medical research. The rats were then randomly divided into four groups of six animals each. Nigella sativa oil was administered orally using a gastric tube.

Plant Acquisition and Preparation:

Nigella sativa seeds were purchased from a commercial shop (Makkah and Madina shop) in Jos metropolis Plateau State, Nigeria. The plant seeds were identified by Mr. Bulus, a taxonomist, Department of Plant Sciences University of Jos, and authenticated by Mr. J.J. Azilla a Botanist from the Federal School of Forestry, Jos. It was given an herbarium voucher number of 0768. The seeds were prepared for phytochemical and elemental analysis, and also oil extraction using Soxhlet method was carried out at the Pharmacognosy Laboratory, Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria. Cyclophosphamide (Cycloxan 500mg), manufactured by Zydus Celexa (Cedila Healthcare Ltd No. 2 ALEAP Industrial Estate, Medical District-500 090 Telengana State, India) was obtained from Le Med Pharmacy, Bauchi Road Jos, Plateau State, Nigeria.

Induction of Gonadal-toxicity:

Cyclophosphamide is an alkylating agent which has been used to treat the different malignant and nonmalignant diseases, it may have some adverse effects including gastro-intestinal disorder, mutagenesis, pulmonary fibrosis, kidney infection, impaired fertility and could induce premature ovarian failure (Meirow et.al., 2014; Jiang et. al., 2013). In this study, Cyclophosphamide is selected to induce ovarian failure as animal infertile models. Therefore, the aim of this study is evaluation of the effect of *Nigella sativa* oil on ovarian structures in Cy-induced ovarian failure in female rats.

Histomorphological Studies of Ovaries and Reproductive Tract:

Tissues were fixed in Bouin's solution and dehydrated using graded alcohol concentrations. This method involved dehydration of tissue in 70% alcohol, 90% alcohol, 95% alcohol, and absolute alcohol, each stage lasting for 30minutes. The use of ascending concentration of alcohol is to prevent the rapid dehydration of tissue thereby causing structural damage to the tissue. The dehydrated tissue is the cleared in two changes of chloroform for 120minutes each. The clearing is to remove the opacity caused by the dehydrating agent, and make the tissue transparent. The tissue is then infiltrated by immersion into molten paraffin for a period of 30minutes. The tissue was then embedded into molten paraffin wax and allowed to solidify. The embedded tissue were blocked in a rectangular block and sectioned using the rotary microtome at 5μm per section. The tissue sections were allowed to float in water bath at 30^{o}C to help the spreading of the tissue ribbons. Clean slides were used to pick the tissues from the warm water bath. The slides were left to dry and later stained using H&E, and PAS stains.

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Table 1. Experimental Group Design.

Experimental Groups	Treatment/Rats (Induced infertility)
Group 1	Positive Control
Group 2	Negative Control
Group 3	200mg/kg NS oil + Cyclophosphamide
Group 4	400mg/kg NS oil + Cyclophosphamide
Group 5	800mg/kg NS oil + Cyclophosphamide

3. Results

Histomorphological Studies:

The estimation of the diameter of follicles, follicular cells and oocytes and the exact calculation of the diameters were ensured by the measuring tools integrated in the Image J software after calibration with a table micrometer (MT12, Heidenhain) (Mandarim-de-Lacerda 2003; Bordbar et al. , 2014). Ovarian follicles at different developmental stages were transplanted according to the methods described by Oktay et al. (2017). In summary, the primary follicles are just below the bark and contain a single layer of scaly grains. Primary follicles consist of a single layer of cuboid granule cells. Secondary follicles contain multiple layers of granule cells, and tertiary follicles are characterized by a granule layer and a fluid-filled antral space.

Table 2. Percentage of rats at different estrus phases from different dose levels of *Nigella sativa* oil. Mean ± S.E.M.

Doses (mg/kg body weight)	**Percentage (Mean ± S.E.M) of rats at different phases of the Estrous cycle**			
	Proestrus	Estrous	Metestrus	Diestrus
Group I (Positive control)	27.06 ± 4.3	25.67 ± 4.2	18.53 ± 2.3	31.23 ± 2.8
Group II (Negative control)	37.03 ± 4.7	27.30 ± 4.7	24.23 ± 2.7	17.36 ± 3.1
Group III (200mg/kg)	47.35 ± 5.3	31. 34 ± 5.3	18.53 ± 7.3	7.52 ± 3.2
Group IV (400mg/kg)	52.07 ± 2.3	36.07 ± 3.2	15.63 ± 2.3	7.20 ± 5.6
Group V (800mg/kg)	50.07 ± 3.2	37.42 ± 4.3	17.73 ± 3.6	9.03 ± 1.8

Mean with different superscript in a row are significantly different when compared with the control group at $p < 0.05$.

Table 3. Weight of the Uterus, fallopian tubes, and Ovaries of Cyclophosphamide induced gonadotoxic rats administrated with *Nigella sativa* oil for 21days across the groups.

Group	Weight of Uterus, fallopian tubes, and Ovaries Mean weight (g) ± SEM	Body weight/Organ ratio Mean weight (g) ± SEM	P- value
Group I (Positive control)	0.58 ± 0.04	231 ± 5.3	0.043
Group II (Negative control)	0.54 ± 0.04	219 ± 4.7^{ab}	0.042
Group III (200mg/kg)	0.56 ± 0.04	227 ± 5.1	0.047

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Group IV (400mg/kg)	0.56 ± 0.04	232 ± 4.9	0.046
Group V (800mg/kg)	0.57 ± 0.06	233 ± 4.0[ab]	0.057

SEM =Standard Error in Mean. [ab] = Statistically significant. Mean with different superscript in a row are significantly different when compared with the control group at $p < 0.05$.

Table 4. Mean serum level of Estradiol and Progesterone cyclophosphamide induced gonadotoxic rats administered with *Nigella* sativa oil for 21 days.

Groups	**Estradiol Mean(pg/ml) ± SEM**	**Progesterone Mean(pg/ml) ± SEM**
Group I (positive control)	7.91± 0.13[ab]	6.31 ± 0.13[b]
Group II (negative control)	6.03 ± 0.17	5.87 ± 0.14
Group III (200mg/kg)	7.41 ± 0.14	5.61 ± 0.09
Group IV (400mg/kg)	7.70 ± 0.13	6.20 ± 0.10
Group V (800mg/kg)	6.76 ± 0.14[a]	7.46 ± 0.14[ab]

P<0.05. S.E.M = standard error in mean. ab = level of significant.

Table 5. Effects of *Nigella sativa* oil on the total volume of ovary, cortex and medulla of Cyclophosphamide induced gonadotoxicity rats.

Groups	**Cortex volume (µm3)**	**Medulla volume (µm3)**	**Total ovary volume (µm3)**
Group I (Positive control)	12.9 ± 2.33	8.01 ± 1.23	20.93 ± 2.1
Group II (Negative control)	10.4 ± 2.11[a]	6.44 ± 0.50[a]	16.79 ± 2.32[a]
Group III (200mg/kg)	12.48 ± 1.46[b]	6.74 ± 1.11[b]	19.22 ± 1.86[b]
Group IV (400mg/kg)	12.77 ± 1.99	8.02 ± 0.89	20.80 ± 1.72[a]
Group V (800mg/kg)	12.53 ± 1.37	8.41± 1.72	19.72 ± 1.62

The data are expressed as the Mean ± Standard Error in Mean (S.E.M) p <0.05.

Table 6. Mean volume of oocyte (µm3) in different types of follicles in different groups of rats 21 days after treatment with *Nigella sativa* oil on induced Cyclophosphamide gonadotoxic rats. Groups (n=6).

	Oocyte volume (µm3)			
	Primordial	**Primary**	**Secondary**	**Antral**
Group I (Positive Control)	1235.93 ± 92.1[a]	2582.08 ± 241.9[a]	4257.44 ± 346.2[a]	1248.01 ± 254.7[a]
Group II (Negative Control)	1252.24 ± 82.1[a]	3579.61 ± 272.7[a]	4196.33 ± 318.7[a]	1752.92 ± 481.3[b]
Group III (200mg/kg)	1357.70 ± 70.7[a]	3595.00 ± 253.5[a]	5775.24 ± 404.0[ab]	1827.00 ± 478.1[a]
Group IV (400mg/kg)	1435.43 ± 71.2[a]	3730.09 ± 335.5[ab]	4587.86 ± 438.5[a]	1594.73 ± 560.1[a]

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Group V (800mg/kg)	1323.43 ± 50.3[a]	3570.09 ± 327.5[a]	4857.86 ± 428.5[ab]	1587.86 ± 310.5[a]

Means with different code letters in each column differ significantly from each other ($p < 0.001$).

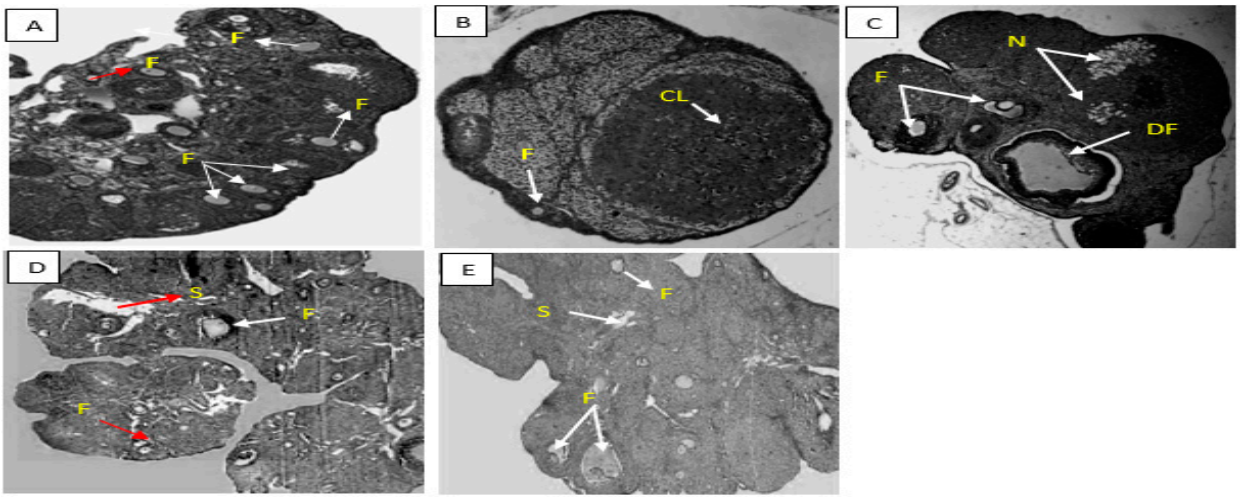

Figure 1. Photomicrograph of Cyclophosphamide induced Ovaries in all experimental groups, after treatment with different concentrations of NS-oil for 21 days. Magnification x 40. A. Group 1 (Positive Control): Ovaries at different developmental stages, with normal histological architecture. B. Group 2. (Negative Control): Shows diminished number of developing follicles and increased number of atretic follicles. C.Group 3. (200mg/kg NS-oil + 0.5 mg/kg Cy): Shows distribution of developing follicles, degenerating follicle and a balloon tissue necrosis on the ovarian surface. D. Group 4 (400mg/kg NS-oil + 0.5 mg/kg Cy): Presence of developing primary follicles and distorted ovarian stroma. E. Group 5 (800mg/kg NS-oil + 0.5 mg/kg Cy): Shows ovary with follicles at different stage of development, and mild necrosis on the surface.

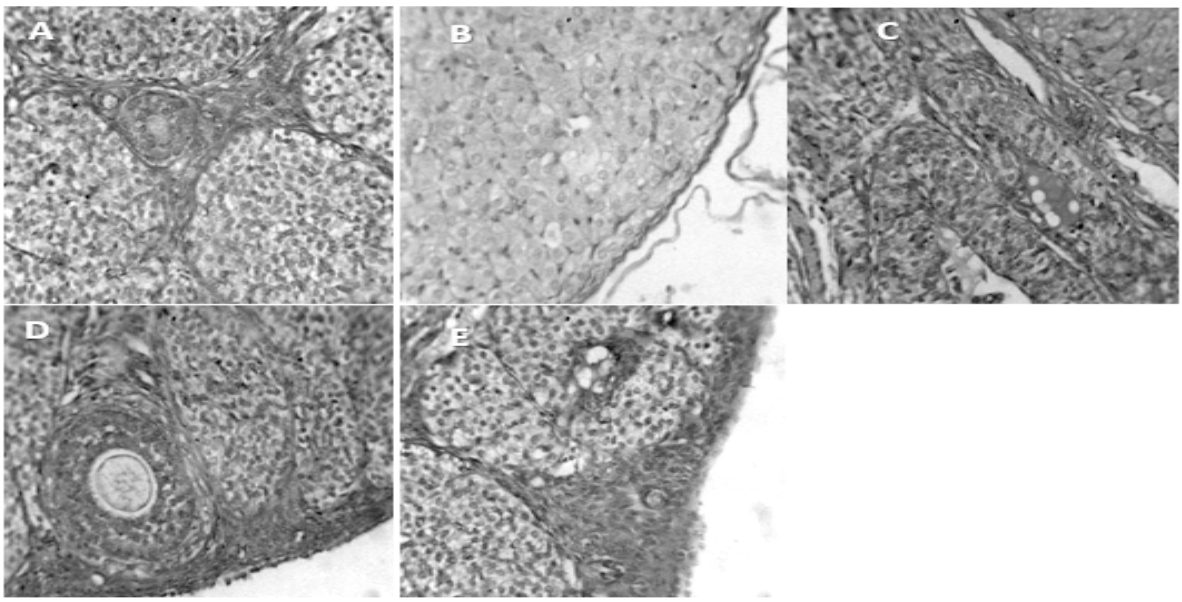

Figure 2. Micrographs showing cross sections of Ovarian Stroma in all treatment groups and the controls. Magnification x100. A. Group 1. (Positive Control). B. Group 2. (Negative Control). C. Group 3. (200mg/kg NS-oil + 0.5mg/kg Cy). D. Group 4. (400mg/kg NS-oil + 0.5mg/kg Cy). E. Group 5. (800mg/kg NS-oil + 0.5mg/kg Cy).

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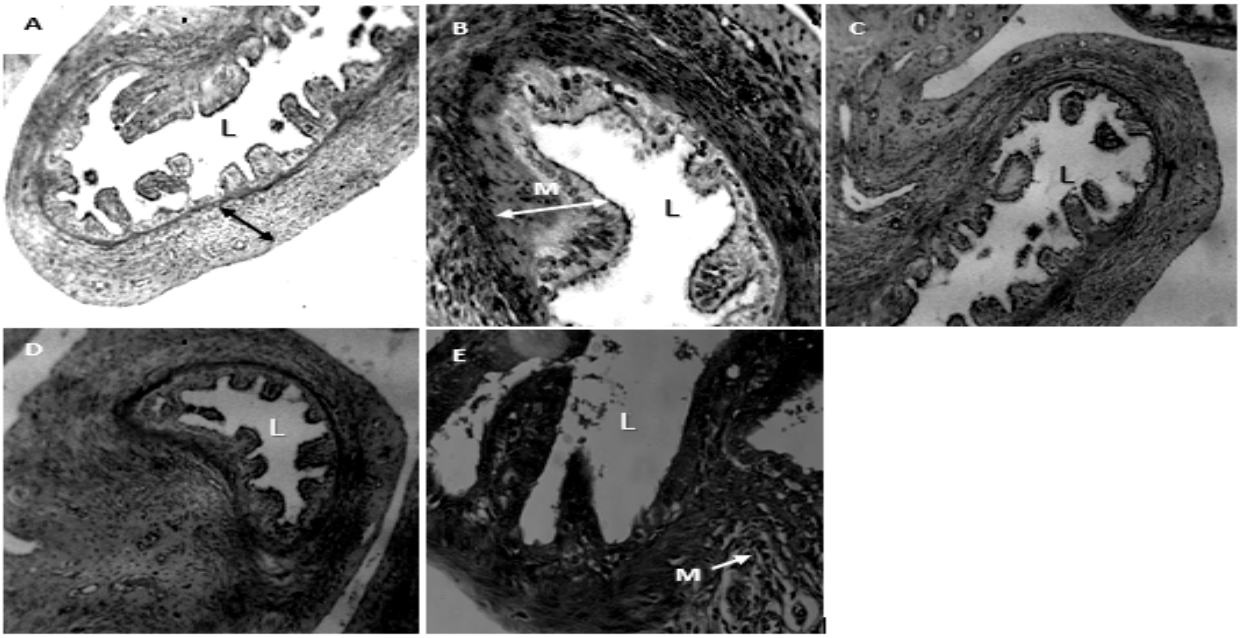

Figure 3. Photomicrographs showing Uterine tubes of experimental rats exposed to 0.5mg/kg Cyclophosphamide and treated with NS-oil of different concentrations for 21days. Magnification x 40. A. Group 1 (Positive Control): Normal appearance of uterine mucous membrane with branching folds, and central lumen (L). B. Group 2. (Negative Control): Shows increased activity of the mucous membrane of the uterine tube. C. Group 3. (200mg/kg NS-oil + 0.5mg/kg Cy), also shows decrease mucous activity, the muscularis mucosa also shows decrease in diameter. D. Group 4. (400mg/kg NS-oil + 0.5mg/kg Cy), Narrowing of the luminal diameter can be seem in this group.

Body and ovarian weight (Table 3). shows that the mean body weight of the animals in all groups had no significant difference. Ovarian weight reduced in the Cyclophosphamide induced experimental groups compared with the control group ($p < 0.05$), but ovarian weight increased after treatment in the Nigella sativa group compared with the control in dose dependent manner ($p < 0.05$). There was no significant difference in these parameters between the experimental and control groups (Table 3).

Volume of the cortex, medulla and total volume of the ovary, and also cortex and medulla volume were reduced in the Cy experimental groups compared with the control group ($p < 0.05$). However, the total volume of the ovary and its cortex were increased after treatment with NS-oil compared with the control experimental groups ($p < 0.05$). There was no significant difference in these parameters between the NS-oil experimental and control groups (Table 5). Number of pre-antral, antral, and atretic follicles Number of pre-antral and antral follicles were reduced in the Cy experimental groups compared with the vehicle group ($p < 0.05$), but pre-antral and antral follicles increased after treatment with NS-oil compared with the Cy experimental groups ($p < 0.05$).

The number of atretic follicles decreased in Cy and NS-oil treated rats compared with the vehicle group ($p < 0.05$). There was no significant difference in these parameters between the NS-oil experimental and vehicle groups (Table 6). Diameter of pre-antral and antral follicles reduced in the Cy experimental groups compared with the vehicle group ($p < 0.001$). The diameter of pre-antral and antral follicle increased after treatment with NS-oil compared with the Cy experimental groups ($p < 0.05$). There was no significant difference in these parameters between the NS-oil experimental and vehicle groups (Table 6).

Diameter of follicular cells in pre-antral and antral follicles Diameter of follicular cells in pre-antral and antral follicle were reduced in the Cy experimental groups compared with the vehicle group ($p < 0.01$) and diameter of follicular cells in pre-antral and antral follicle were increased after

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treatment with NS-oil related to the Cy experimental groups ($p < 0.05$) and decreased compared with the vehicle.

Diameter of oocyte in pre-antral and antral follicles Diameter of oocyte in pre-antral follicle was reduced in Cy and NS-oil experimental groups compared with the vehicle group ($p < 0.05$). Diameter of oocyte in antral follicle was reduced in the Cy and NS-oil experimental groups compared with the vehicle group ($p < 0.05$). There was no significant difference in this parameter between the other experimental groups (Table 6)

4. Discussion

The rat has a brief reproductive cycle of 4 to 5 days in phases, which makes it excellent for reproductive investigations (Campinas & Campinas, 2002; Caligioni, 2009). The release of gonadotropin-releasing hormone from the brain, gonadotropins from the pituitary, and sex hormones from the gonads all impact the estrous cycle in sexually developed female animals (Choi et al., 2016; Nie et al., 2021). Plant extracts inhibited the estrous cycle in rats during the diestrus phase, preventing the production of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Ojah & Borthakur, 2021; Oluwasola et al., 2019).

The loss of regular cyclicity implies that the ovarian progestin and estrogen equilibrium has been disrupted. Studies reported that many extracts resulted to lengthened the diestrus stage of the cycle (Hubscher et al., 2005; Regulation, 2021), and sometimes complete abolition of proestrus, with prolonged estrus, and shortened diestrus phases of the cycle following the administration of *Nigella sativa* oil (Bridgewater et al., 2020; Uzochukwu, 2020). The results of the current study (Table 2) confirm these previous claims of the ability of some plant extracts to lengthen the diestrus phase of the cycle. Distinct plants have different ways for altering the reproductive cycle, as evidenced by the submissions.

The result in this studies shows that there was an increase in estradiol hormone and progestin in the rats that were treated with 800mg/kg *Nigella sativa* oil (Table 4), this is in line with several studies of *Nigella sativa* oil on reproduction, showed that it stimulated the secretion of sexual hormones that led to improve synthesis of hepatic enzymes (Elkareem et al., 2021; Hannan, Zahan, et al., 2021), it increases the thickness of germinal layer of seminiferous tubules significantly (Al-zuhairy, 2014; Al Taee, 2018), also reverse toxicity effect in rats treated with Lead acetate (100mg/kg), and causes significant enhancement in reproductive function, increases ovarian weight to body ratio, gonadotrophins, and diameter of graafian follicles (Bala, et. al., 2020; Hannan, Rahman, et al., 2021)

Ovarian failure is distinguished by ovarian atrophy, follicles reduction, and sex hormonal diminution (Ebrahimi and Asbagh 2011). Cyclophosphamide is an alkylating agent which induce ovarian failure in animal models (Budel et al. 1988; Comish et al. 2014). This quantitative study showed that Cy decreased weight and volume of ovary, reduced different follicles number and sex hormones levels and increased atretic follicles compared to vehicle group. Reduction of the medulla volume may be attributed to the rate of blood circulation. suggesting that, reduction of the rate of medulla blood circulation caused to medulla volume diminution. Reduction of cortex volume can be related to follicular number decrement. This result indicates that Cy had the most important effect on the number of pre-antral follicles relative to antral follicle and increase atretic follicle. Comish et al. (2014) showed that Cy causes degeneration of ovarian follicle number in mice. Other studies have demonstrated that Cy significantly reduces primordial and primary follicles and increases the numbers of atretic follicles (Budel et al. 1988; Comish et al. 2014).

This result also shows that Cyclophosphamide adversely affect follicular development at all stages of development. This is in line with Epstein (1990), who believed that Cy has negative effect on both resting and dividing cells. Lopez and Luderer (2014) show that Cy leads to misdirection of the diakinesis stage in first meiosis of oogonia. Other researches have demonstrated that Cy inhibits follicular cell division through DNA strand break and inhibit mitotic divisions (Mills et.al, 2018; Sun et. al., 2021). Based on this information, the other parameters should be measured to confirm this data. In this research, cyclophosphamide reduced the diameters of different follicles, oocyte and follicular cells relative to vehicle group. Lopez and Luderer (2004) demonstrated that Cy-induced

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follicular cell apoptosis in rats. Other studies showed that ovarian failure had the most side effect on follicular cells compared with resting follicle (Jankwoska 2017; Huang et. al., 2022). Researches show that, any factor which induces follicle syncope in the primary follicle stage, inhibit follicular cell mitosis, consequently, follicular cell remain only one layer and formation of any new layers of follicular cells are uncommon (de Castro et al. 2016; Yang et. al., 2017). It seems reduction of follicular cell in pre-antral follicle prevent germ cell development and decrease the number of pre-antral and antral follicles. In addition, reduction of follicular cells has direct influence on the size of oocyte in growing follicle.

This study also demonstrated that Cy decreased the level of estradiol and progesterone. Follicular cells are the main source of estradiol and progesterone. Destruction of antral and pre-antral follicles have a direct effect on estradiol level (Holesh, 2022). Therefore, it seems that change in the number of the growing follicles influences the level of hormones. Studies show that growth factors have an important role in improving the structure and function of ovaries. Different growth factors such as CTGF, EGF, and TGF-b have protective effects on ovarian damage (Yan et al. 2001; Su et al. 2008; Uhm et al. 2010; Nagashima et al. 2011; Mendes et al. 2015). In this study, it was found that NS oil had a positive effect on the volume of the ovarian cortex, the number of preantral follicles, and the diameter of the antral follicles. These results also show that NS oil increases the volume of the ovarian cortex, the number of preantral follicles, and the diameter of the antral follicle and its ovule.

Based on these results, it can be proposed that NS-oil accelerates antral folliculogenesis by stimulating follicular cell proliferation and thereby increasing antral follicle diameter. An increase in oocyte diameter in the antral follicles under the influence of growth factors is a common phenomenon, while a decrease in oocyte diameter in the preantral follicles under this condition is an unusual phenomenon. The mechanism of cell size is not fully understood but is determined by external and internal factors (Marshal et. al., 2012; Lloyd 2013). Zetterberg et al. (1984) believe that macromolecular factors such as EGF have a stimulatory effect on DNA synthesis but do not affect cell size. In addition, GDF9 stimulates meiotic division in metaphase I and II and regulates follicle and ovarian size (Comish et al. 2014; Da Broi et. al., 2018). In this study, the extract oil of NS-oil had no effect on estradiol and progesterone levels. The follicular cells do not appear to have reached their full development; Therefore, the levels of these hormones have not changed significantly. In this study, we also found that NS-oil administration had no effect on various ovarian structures and functions in normal rats. It appears that the beneficial effects of NS-oil apply only to damaged ovaries and do not affect normal structure. Conclusively, Cyclophosphamide had a more damaging effect on follicular cells than on oocytes in different ovarian follicles. NS-oil had a positive effect on follicle growth and improved ovarian tissue repair.

5. Conclusions

The results of this investigation provide light on the intricate interactions that occur between plant extracts of NS oil, in particular and reproductive physiology in rat models, especially when cyclophosphamide (Cy) is present. The study demonstrated that exogenous chemicals and innate physiological processes have a substantial impact on the short estrous cycle in female rats.

Cy treatment caused significant ovarian dysfunction, which included ovarian shrinkage, a drop in the number of follicles, and low sex hormone levels, especially progesterone and estradiol. This is consistent with other research showing that Cy causes ovarian failure by means of follicular cell death and DNA damage, which ultimately results in abnormal folliculogenesis and hormonal abnormalities. These results are supported by the study's quantitative analysis, which shows a notable rise in atretic follicles and a decrease in the volume of the ovarian cortex and medulla, both of which are essential for healthy reproductive function.

On the other hand, NS oil administration showed ovarian structures to be protected, increasing the number of preantral follicles and the diameter of antral follicles, indicating that it may have a role in folliculogenesis and ovarian tissue healing. The fact that NS oil treatment raised estradiol and progesterone levels added to the evidence supporting its effectiveness in promoting ovarian function, especially in impaired situations brought on by Cy.

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These findings highlight the significance of comprehending the processes by which NS oil extracts can alter hormone levels and reproductive cycles, laying the groundwork for future therapeutic uses in the treatment of reproductive dysfunctions. Future studies should concentrate on clarifying the precise mechanisms by which Nigella sativa oil works to cure ovarian-related diseases and investigating the oil's potential use in clinical settings. All things considered, this research offers insightful information about the dynamics of reproductive health and the possibility that natural substances can lessen the negative effects of synthetic medications like cyclophosphamide.

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