




# Benefits of *Nigella sativa* Extract Protecting Ovary Due to Cisplatin Chemotherapy

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## Abstract

**BACKGROUND:** Cisplatin (CIS) is an important chemotherapy agent which is widely used for the treatment of many types of solid tumors, which can cause decreased ovarian function. *Nigella sativa* has been shown to have an anti-inflammatory and anti-oxidant activity that might protect the ovaries from damage due to CIS.

**AIM:** This study aims to understand the benefits of *N. sativa* protecting the ovaries due to CIS chemotherapy.

**METHODS:** Thirty-two female *Rattus norvegicus* aged 8 weeks weighing 160–200 g were divided into four groups: Negative control, Positive control, Treatment-1 (CIS 6 mg/KgBW and NS 500 mg/KgBW/day), and Treatment-2 (CIS 6 mg/KgBW and NS 1000 mg/kgBW/day) for 2 weeks. On the 14<sup>th</sup> day the rats were sacrificed, blood was drawn from the heart, followed by taking ovaries.

**RESULTS:** There was lower mortality and morbidity in CIS + NS 1000 and CIS + NS 500 mg group ( $p = 0.01$  and  $0.001$ ). The mean estradiol levels, follicle-stimulating hormone levels, and anti-mullerian hormone levels were not statistically significant among the four groups. The highest number of primary, secondary, tertiary follicles are seen at the CIS + NS 500 mg and CIS + NS 1000 mg groups ( $p = 0.05$ ). The lowest number of atretic follicles is seen at the CIS + NS 1000 mg group, and the highest number of atretic follicles was in CIS only.

**CONCLUSION:** There is a trend that *N. sativa* is beneficial in protecting the ovaries from damage caused by CIS.

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## Introduction

Artificial or acquired ovarian function decline, among others, occur in cancer patients treated with radiation and chemotherapy. According to Globocan in 2018, there were 18.1 million new cases with a death rate of 9.6 million, where one in five men and one in six women in the world experience cancer [1]. Top five female cancers in Indonesia: Breast 65,858 (30.8%), cervix. 36,633 (17.2%), Ovary 14,896 (7%), Colorectum 12,425 (5.8%), and Thyroid 9,053 (4.2%) [2].

The survival of women with various types of cancer has improved significantly with improvements in cancer treatment, including early detection and effective management [3]. The American Cancer Society reports in Cancer Facts and Figures, that the 5-year relative survival for all cancers has increased substantially since the early 1960s, from 39% to 70% among whites and from 27% to 63% among blacks.

Increased survival of women with cancer who need quality of life care, which preserves their fertility. Cisplatin (CIS) and Paclitaxel are the most widely used chemotherapeutic agents for the treatment of gynecological malignancies, and their mode of action against ovarian cancer cells has been thoroughly investigated. CIS interferes with DNA replication and kills the fastest-growing cells [4]. The effects of cancer and its treatment on female reproductive function are increasingly well documented. Overall, compared to the general population, women were 38% less likely to become pregnant after diagnosis and undergoing cancer treatment, in all cancer diagnostic groups being associated with a decreased likelihood of subsequent pregnancies [5]. Given this situation, there is now an urgent need to develop methods to protect the ovaries from the deleterious effects of treatment, thereby reducing the risk of decreased fertility and premature menopause. CIS-induced ovarian damage has been found in studies of the culture of cortical pieces of

human ovaries or granulosa cells, found a decrease in the number of follicles and a reduction in steroidogenic activity [6], [7].

There is a lot of evidence showing the loss of ovarian reserves and increased follicular atresia after exposure to CIS in the ovaries of rats and mice [7], [8], [9], [10], [11], [12], CIS is an anti-drug. cancers that cause ovarian damage in a moderate category [13].

Chemotherapy and radiation agents have been shown to induce oxidative stress and inflammation. Four different antioxidants, Mesna, Mirtazapine, Resveratrol, and Sildenafil citrate, have been tested for their protective effect on ovarian reserves of CIS-treated mice [14], [15], [16]. Mesna, Sildenafil citrate, and Resveratrol prevent anti-mullerian hormone (AMH)-positive follicle loss; Mirtazapine also increases fertility. The enzymatic antioxidant activity of superoxide dismutase (SOD) and glutathione increased after administration of Mesna or Mirtazapine with CIS.

Assi et al. (2018) reported, giving *Nigella sativa* to experimental mice that experienced a decrease in ovarian function due to lead acetate showed a significant increase in ovarian function compared to controls. *N. sativa* containing thymoquinone (TQ) monoterpene has been shown to exhibit strong antioxidant properties and a protective effect against oxidative damage caused by some drugs and toxins. CIS treatment causes an oxidant/antioxidant imbalance which is reflected in an increase in lipid peroxidation, a decrease in enzymatic and non-enzymatic antioxidants [17].

Seeing the results of previous research on the benefits of *N. sativa* in improving ovarian function, and seeing the ovarian damage caused by chemotherapy, it is necessary to conduct research on the benefits of *N. sativa* containing TQ to protect ovarian function in women undergoing chemotherapy, starting with research on experimental mice.

## Methods

### **Research type and location**

This experimental animal research was carried out at the Animal Laboratory of the Faculty of Mathematics and Natural Sciences Sumatera Utara University. Estradiol, AMH, and follicle-stimulating hormone (FSH) level examination of ovaries was carried out at the Integrated Laboratory of Faculty of Medicine, Sumatra Utara University.

Histological examination of the ovary was carried out at the Department of Anatomical Pathology, Faculty of Medicine, Sumatra Utara University.

### **Material**

1. *N. sativa* seed extract in the form of oil, brand Habbasy 500 mg and 1000 mg
2. CIS
3. Ovary
4. Blood.

### **Experimental animal**

The protocol of the study was approved by the Animal research ethics committee of the Faculty of Mathematics and Natural Sciences Sumatera Utara University with reference number of 00714/KEPH-FMIPA/2020 in accordance to "Guide for care and use of laboratory animals" set by the Faculty of Mathematics and Natural Sciences, University Sumatera Utara.

Thirty-two female laboratory rats (*Rattus norvegicus*) aged 8 weeks weighing 160–200 g were used in this study, according to Federer's formula (1963), Rats were acclimatized for 7 days, given food and drink *ad libitum*. They were placed in a cage in a room with a temperature of 22–25°C with a light-dark cycle of 12/12 h. Wistar rats were divided into 4 groups: (1) C1 = Negative control (rats were not given CIS). (2) C2 = Positive control (rats were given an injection of single dose CIS 6 mg/kgBW intraperitoneally only). (3) T1 = Treatment 1 (rats were given a single dose of CIS 6 mg/KgBW intraperitoneal and NS 500 mg/KgBW/day). (4) T2 = Treatment 2 (rats were given a single dose of CIS 6 mg/KgBW intraperitoneally and NS 1000 mg/kgBW/day) for 2 weeks. On the 14<sup>th</sup> day the rats were sacrificed, blood was drawn from the heart, followed by taking ovaries.

### **Blood collection**

Prior to blood sampling, the rats were anesthetized with diethyl ether to ease handling. The blood samples were collected by cardiac puncture using 25G, 1" needle. Approximately 2–3 ml of blood samples were taken and dispensed into labeled plain tubes. The blood samples were then centrifuged at 3000 rpm for 10 min to separate the sera. The serum was stored at –80°C until further use.

### **Ovarium histological study**

The ovary was removed and freed from all connective tissue prior to wet weight recordings. Routine histological processes were employed and each ovary was cut. Each portion was prepared as a block and randomly chosen and examined under a light microscope (Olympus CK2) for primary, secondary, tertiary, and atretic follicles. Appropriate image capture was done using a light microscope (Olympus CK2) coupled to a camera (Olympus BX 41).

### Chemicals and reagents

Estradiol, FSH, and AMH levels were examined by enzyme-linked immunosorbent assay (ELISA), using rat Estradiol ELISA Kit, 96T rat reagent, rat FSH ELISA Kit 96T, and rat AMH ELISA Kit 96T from Elabscience. Histopathological examination was carried out by using Hematoxylin Eosin staining.

### Statistical analysis

To assess whether the sample is normally distributed or not, the Shapiro-Wilk test is carried out because the sample is  $<50$ . Data are presented in the form of mean standard deviation. To assess the comparison of parameters between the control group and the treatment group, the One Way analysis of variance (ANOVA) and Kruskal's Wallis test analysis were used. Data were processed and analyzed using SPSS with a significance limit of  $p < 0.05$ .

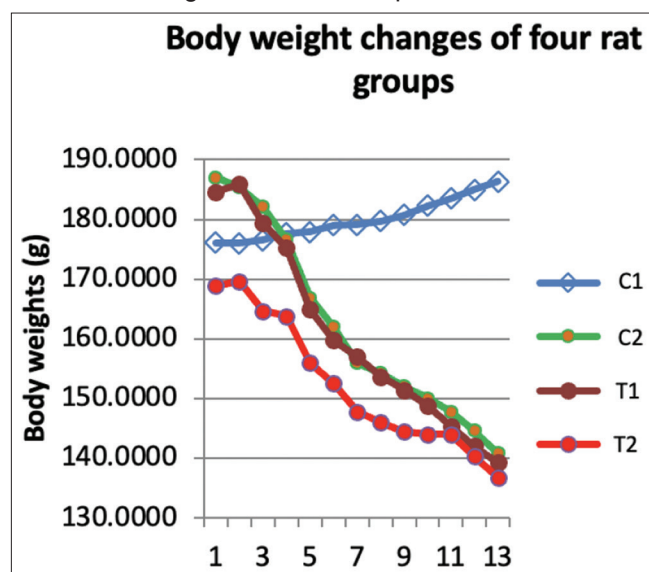


Figure 1: Comparison of mean body weight before and after intervention in 4 groups

## Results

### Mortality and morbidity

There were 4 (50%) deaths of rats in the CIS group, 2 rats died on the 7<sup>th</sup> day and 2 on the 8<sup>th</sup> day. In CIS + NS 500 mg, 2 dead rats (25%), both of which died on the 9<sup>th</sup> day. There were no rat that died in the Negative Control and CIS + NS 1000 mg group ( $p = 0.01$ ,  $<0.05$ ). Four rats that died in the CIS group and 2 in the CIS + NS 500 mg group were then replaced with other rats and given the appropriate treatment. There were no health problems that occurred in the negative control group rats, while the positive control group that received CIS all experienced morbidity of diarrhea, nosebleeds, and eyesores (100%). The rats in the CIS + NS 500 mg

group and CIS + NS 1000 mg) had diarrhea, nosebleeds, eyesores as much as 50% and 25%, respectively. The morbidity score differences in four groups of study was significant statistically ( $p = 0.001$ ,  $p < 0.05$ ).

Results of weight assessment before treatment and 14 days after treatment showed a significant reduction in body weight ( $p < 0.05$ ) (Figure 1). This weight loss was due to the effects of CIS chemotherapy, while the Negative control group who did not receive CIS gained weight.

### Estradiol, FSH, and AMH levels

The lowest mean level of estradiol was found in the CIS group, namely  $66.97 \pm 20.45$  pg/ml, the highest mean level was found in the CIS + NS 1000 group, namely  $114.53 \pm 35.33$  pg/ml. There was a decrease in Estradiol levels in the CIS group compared to Negative Control and an increase in Estradiol levels in the CIS + NS 500 and CIS + NS 1000 groups compared to CIS only, ( $p = 0.069$ ,  $p > 0.05$ ), meaning that statistically there was no significant difference (Figure 2).

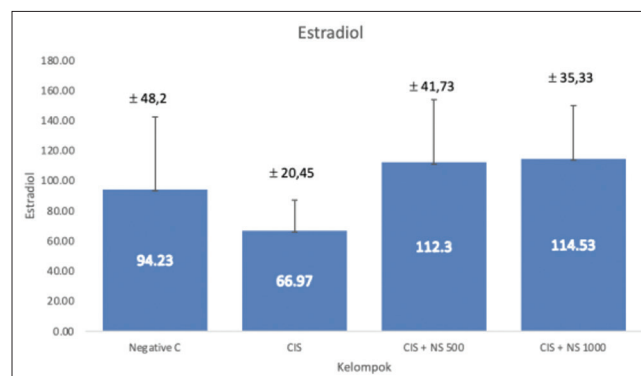


Figure 2: Diagram of mean and standard deviation of Estradiol levels ANOVA,  $p = 0.06$

The lowest mean of FSH levels was in the Negative control group, namely  $19.49 \pm 2.64$ , the highest mean level was in the CIS + NS 500 groups, namely,  $21.68 \pm 0.92$  ng/l. There was an increase in FSH levels in the CIS group compared to Negative control, and a decrease in FSH levels in the CIS + NS 1000 group compared to CIS, but the value of  $p = 0.069$  ( $p > 0.05$ ), meaning that statistically there was no significant difference (Figure 3).

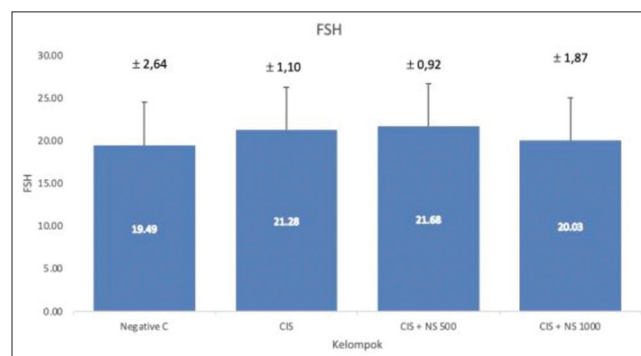


Figure 3: Diagram of mean and standard deviation of follicle-stimulating hormone levels ANOVA,  $p = 0.06$

The lowest mean AMH levels were in the CIS group, namely  $3.63 \pm 2.11$  ng/ml, the highest mean levels were in the Negative control group, namely,  $4.54 \pm 3.53$ . Statistical tests with ANOVA, the p-value was 0.932 ( $p > 0.05$ ), meaning that there was no statistically significant difference (Figure 4).

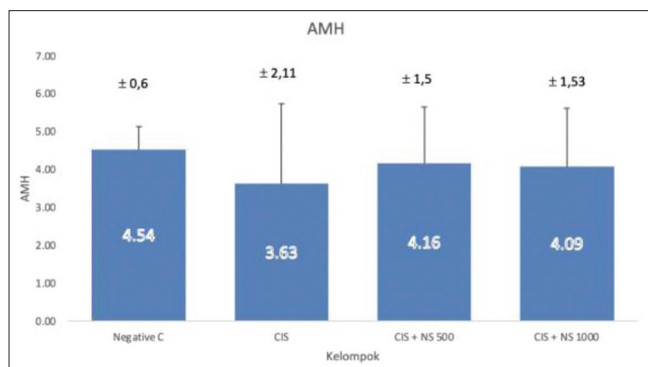


Figure 4: Mean and standard deviation of anti-mullerian hormone levels ANOVA,  $p = 0.93$

Because there were no significant differences in the levels of estradiol, FSH, and AMH, to see the superiority between the treatment groups, it could be seen the difference in the value of each treatment group against the positive control (Table 1).

Table 1: Comparison of the difference in levels of Estradiol, FSH, and AMH to CIS only group

Group	C1	C2	T1	T2	ΔD T1-C2	ΔD T2-C2
Estradiol (pg/ml)	94.23	66.05	112.30	114.53	46.25	48.48
FSH (ng/ml)	19.49	21.28	21.68	20.03	0.4	-1.25
AMH (pg/ml)	4.54	3.63	4.16	4.09	0.53	0.46

FSH: Follicle-stimulating hormone, AMH: Anti-mullerian hormone, CIS: Cisplatin.

It can be seen that in the CIS + NS 500 and P CIS + NS 1000 groups, the mean levels of estradiol are higher than those of CIS only without NS, with a difference of an increase of 46.25 pg/l and 48.48 pg/l, it appears that CIS + NS 1000 is slightly superior to CIS + NS 500. This is in line with the lower in FSH levels in the CIS + NS 1000 group compared to CIS only and higher AMH levels in the CIS + NS 500 and CIS + NS 1000 groups

Table 2: Comparison of the difference in primary, secondary, tertiary, and atresia follicles count to CIS only group

Group	C1	C2	T1	T2	ΔD C2-T1	ΔD C2-T2
Primary, secondary, tertiary, follicles	6.87	7.37	16.62	31.50	9.25	24.25
Atresia follicles	23.0	44.62	33.37	16.87	-11.25	-27.75

.CIS: Cisplatin.

### Ovarian histopathology examination results

Results of the histopathology examination are shown in (Figures 5-8, and Table 2).

## Discussion

The cause of death in four mice that received CIS alone and two mice in the CIS + NS 500 mg group,

is not known with certainty, it should be suspected that death was due to the toxic effects of CIS on organs and serious gastrointestinal disorders such as severe diarrhea, nausea, vomiting, and reduction appetite and dehydrated. The majority of morbidity in this study was diarrhea (75%).

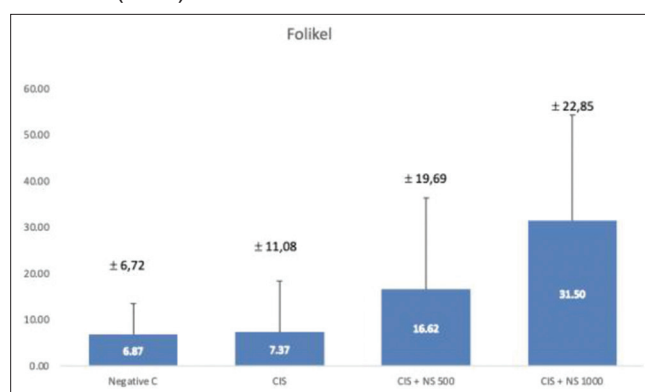


Figure 5: Mean and standard deviation of primary, secondary, tertiary follicle count Kruskal Wallis,  $p = 0.05$

Based on the results of this study, giving *N. sativa* seems to reduce mortality and morbidity of mice due to CIS, but does not prevent weight loss. Weight loss was found in all animal groups ( $p < 0.05$ ). This seems to be related to the side effects of CIS. Aldossary (2013) reported the results of his study on 123 patients with 11 types of tumors that the majority of patients (72%) experienced gastrointestinal disorders, such as nausea and vomiting, diarrhea, constipation, epigastralgia, pyrosis, dysphasia, postprandial abdominal bloating sensation, tongue white, dysgeusia and taste disorders. Among other side effects, constitutional symptoms include hypostenia and asthenia, fever, weight loss, and decreased appetite. Nausea and vomiting are considered to be the most common types of CIS poisoning during chemotherapy. One study examining the toxicity of CIS after a dose of  $120 \text{ mg/m}^2$  found that patients who did not receive antiemetic drugs prior to CIS had an average of 11 episodes of emetics [18]. The presence of this prolonged nausea and vomiting, coupled with diarrhea may be associated with decreased appetite, and ultimately lead to weight loss.

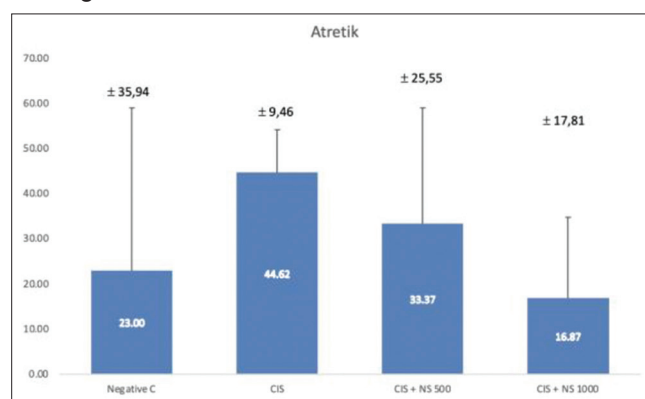


Figure 6: Mean and standard deviation of atresia follicle count Kruskal Wallis,  $p = 0.31$



Cachexia, an accidental weight loss of  $\geq 5\%$ , can occur as a serious and dose-limiting chemotherapy side effect that decreases the survival of cancer patients. Changes in lipid metabolism are thought to cause the lipodystrophy commonly associated with cachexia. Ghrelin has been proposed to correct changes in lipid metabolism due to its orthogenic and anabolic properties. There are studies showing the effects of CIS and Ghrelin on lipogenesis, but not on lipolysis and  $\beta$  oxidation. Thus, Ghrelin prevents CIS-induced weight and fat loss by restoring adipose tissue function. The increase in calorie intake further increases the anabolic effect of Ghrelin [19].

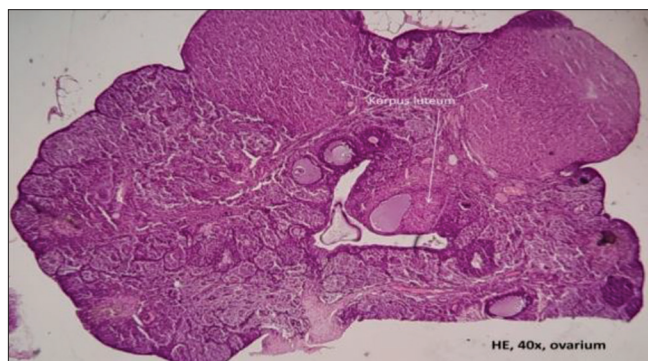


Figure 7: Ovary at 40 $\times$  magnification

The results of this study showed that statistically there was no significant difference in the levels of estradiol, FSH, and AMH in the four study groups, so to know the benefits of *N. sativa* and to see the advantages between treatment groups can be seen the difference in value from each treatment group to the positive control. The lowest mean of estradiol levels was found in the CIS group, namely  $66.05 \pm 20.45$  pg/ml, while the mean estradiol levels in the negative control group were  $94.23 \pm 48.20$  pg/ml. There was a decrease in the mean of estradiol levels by 28.28 pg/ml from the normal value, meaning that there was suppression of ovarian function in the rats receiving CIS 6 mg intraperitoneally. There was an increase in estradiol levels in the CIS + NS 500 and CIS + NS 1000 mg groups compared to the CIS group, namely,  $112.30 \pm 41.73$  pg/ml and  $114.53 \pm 35.33$  pg/ml with a difference of 46.25 pg/l and 48.48 pg/l. The increase in the mean of Estradiol in the CIS + NS 500 mg and CIS + NS 1000 mg groups even exceeded the normal Estradiol values as found in the Negative control group, namely  $94.23 \pm 48.20$  pg/ml, this means that the administration of *N. sativa* at a dose of 500 mg/KgBB and 1000 mg/KgBB po/days able to prevent mouse ovarian suppression due to receiving CIS 6 g/kgBW intraperitoneally. The administration of *N. sativa* at a dose of 1000 mg/KgBW is better than the dose of 500 mg/KgBB.

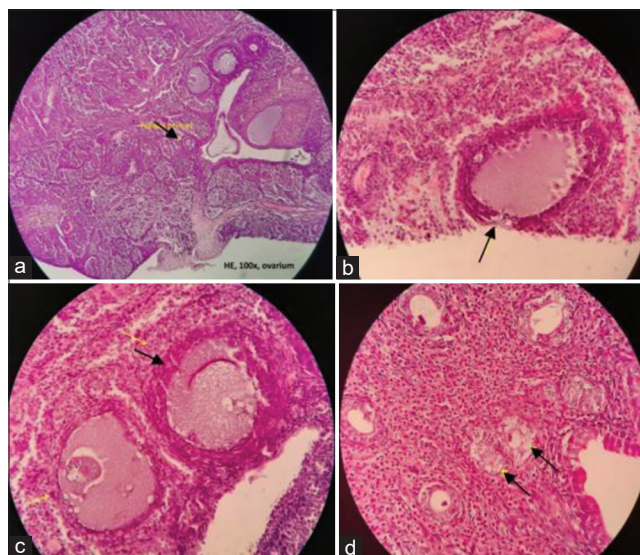


Figure 8: Primary, secondary, tertiary, and atretic follicles of *Rattus norvegicus* (a) Primary follicle, magnification 100; (b) Secondary follicle, magnification 400 $\times$ ; (c) Tertiary follicle, magnification 400 $\times$ , (d) Atretic follicle, magnification 400 $\times$ )

The mean FSH levels increased in the CIS group, namely  $21.28 \pm 1.11$  ng/l, while the mean FSH levels in the Negative control group were  $19.49 \pm 2.64$  ng/l. There was an increase in the mean FSH level of 1.78 ng/l from the normal value. The increase in FSH levels is a response from the hypothalamus and pituitary caused by low levels of estradiol produced in the ovaries, which is referred to as negative feedback. This means that there is suppression of ovarian function in rats receiving CIS 6 mg/KgBW intraperitoneally. There was a decrease in FSH levels in the CIS + NS 1000 group but not in CIS + NS 500 compared to the Negative control, namely,  $20.05 \pm 1.87$  ng/ml with a decrease difference of 1.23 ng/ml which is almost close to the normal value. This means that giving *N. sativa* at a dose of 1000 mg/KgBB po/day is useful for preventing ovarian suppression of rats due to receiving CIS 6 mg/KgBB intraperitoneally.

The lowest mean of AMH levels was found in the CIS group, namely,  $3.63 \pm 2.11$  ng/ml, while in the Negative control group was  $4.54 \pm 0.60$  ng/ml. There was a decrease in the mean AMH level of 0.91 ng/ml from the normal value of AMH. A decrease in AMH levels, although not statistically significant, indicated that there was a suppression of ovarian function in mice receiving CIS 6 mg/kg intraperitoneally.

Abdulah *et al.* (2014) reported that there was a significant decrease in the median serum level of AMH and Inhibin B patients between before and after Paclitaxel-CIS chemotherapy [20]. There was a decrease in the median AMH value from 2.54 ng/ml to 1.99 ng/ml after the first chemo ( $p = 0.01$ ), to 1.49 ng/ml after the second chemo ( $p = 0.001$ ), and to 1, 37 ng/ml after the third chemo ( $p = 0.000$ ,  $p < 0.05$ ). Likewise, the median decrease in serum AMH and Inhibin B levels after Paclitaxel-CIS chemotherapy in each chemotherapy series  $p = 0.000$  ( $p < 0.05$ ). Conclusion:

AMH and inhibin B serum levels in cervical cancer patients who received combination chemotherapy decreased dramatically after 3 months of chemotherapy and the factor that contributed to the decrease was age.

It was found that the number of primary, secondary, tertiary follicles was higher in the CIS + NS 500 and CIS + NS 1000 groups, namely,  $16.625 \pm 19.697$  and  $31.5 \pm 22.859$  compared to the Negative control and CIS group, namely,  $6.875 \pm 6.728$  and  $7.375 \pm 11.083$  with a difference of 9.25 and 24.25, where the largest difference is in CIS + NS 1000 ( $p = 0.05$ ).

This is in line with the low average number of atretic follicles in the CIS + NS 500, namely  $16.87 \pm 17.81$ , and CIS + NS 1000 ( $33.37 \pm 24.55$ ) compared to CIS group ( $44.62 \pm 9.46$ ) and there are the difference  $-27.75$  and  $-11.25$  to CIS group. Based on the delta of the difference in the mean number of primary, secondary, tertiary, and atretic follicles, CIS + NS 1000 is superior to CIS + NS 500. This suggests that *N. sativa* is beneficial to prevent decline and even improve ovarian function in rats given the chemotherapy drug CIS.

In this study, the decrease in estradiol and AMH levels was not as large as in other studies, possibly because the dose of CIS used in this study was single dose of 6 mg/KgBW, and the assessment of ovarian function was carried out after 14 days, while in other studies using a dose of 7.5 mg/KgBB and 5 mg/KgBB 2 $\times$  with intervals of 5–7 days and assessment of ovarian function is carried out in 7–10 days.

CIS-induced ovarian damage has been found in studies of the culture of cortical pieces of human ovaries or granulosa cells, found a decrease in the number of follicles and a reduction in steroidogenic activity [6], [7]. There is evidence showing a loss of ovarian reserve and an increase in follicular atresia after exposure to CIS in the ovaries of mice and mice [7], [8], [9], [10], [11], [12], immature oocytes are very vulnerable [9], [10]. Studies examining the effects of CIS exposure on the follicle often report loss of PMF, through direct and or indirect death of PMF due to accelerated activation, ultimately leading to premature ovarian insufficiency [21].

Four different antioxidants, Mesna, Mirtazapine, Resveratrol, and Sildenafil citrate, have been tested for their protective effect on the ovarian reserve of CIS-treated mice [14], [15], [16]. Mesna, Sildenafil Citrate, and Resveratrol prevent AMH-positive follicle loss; Mirtazapine also increases fertility. The enzymatic antioxidant activity of SOD and Glutathione increased after administration of Mesna or Mirtazapine with CIS. These results indicate that keeping free radicals low in the ovaries is important for increasing the survival of ovarian reserves. Almost all journals report research results that CIS destroys granulosa cells by the occurrence of oxidative stress which depletes ovarian reserves and some products can protect ovarian reserves through anti-oxidation.

*N. sativa* has been shown to have strong anti-oxidant properties. This potent anti-oxidant activity is reflected in the strong free radical scavenging action against superoxide anions and enhancing transcription genes responsible for the production of natural anti-oxidants such as SOD, Catalase, and Glutathione peroxidase [22].

*N. sativa* used in this study is Habbasyi Oil which contains *N. sativa* extract in the form of 500 mg oil in each capsule. The results of the phytochemical laboratory test showed that Habbasyoil contained TQ.

Apart from the antioxidant activity that can protect the ovaries, Mohammed (2013) found estrogen (ER)-like activity of *N. sativa* extract in female mice, which is assumed to stimulate follicle development and the formation of corpora lutea. Female rats treated with *N. sativa* extract showed an increase in the total concentration of serum protein and the hormone progesterone [23]. Apart from TQ, *N. sativa* also contains saponins which can accelerate the formation of new blood vessels in the wound healing process (angiogenesis) through VEGF. Zinc or zinc in *N. sativa* is also needed in healing ovarian damage due to CIS [24].

Based on the results of the analysis of Estradiol, FSH, AMH, and the number of antral ovarian follicles in this study, this study showed a tendency to protect the ovaries from damage due to chemotherapy CIS by *N. sativa* without reducing the anticancer effectiveness of CIS. There is a great deal of research on TQ, alone or in combination with other major chemotherapy agents, which can significantly inhibit cancer progression and synergistically reduce the tumor burden on various malignancies through alteration of multiple tumorigenic pathways.

Ganji-Harsini *et al.* (2016) conducted an *in vitro* study on breast cancer cells reported that treatment with a combination of tamoxifen (TAM) and TQ significantly decreased cell viability compared to TAM alone [25]. The apoptotic index increased significantly in the TQ 150  $\mu$ M ( $p < 0.05$ ), TAM ( $p < 0.01$ ), TQ 150  $\mu$ M + TAM ( $p < 0.001$ ) groups compared to the control group. The synergistic effect of TQ and TAM agents on ER + and ER cells suggests that TQ functions via an ER-independent pathway. Investigations revealed that TAM induces apoptosis in ER + cells via a nitric oxide-dependent pathway. The use of high doses of TQ can reduce the dose of TAM and the duration of administration in the treatment of ER + and ER breast cancer cells. Since the number of necrotic cells did not increase in the TQ-treated group, it is safe and harmless for the treatment of resistant metastatic breast cancer patients.

Based on *in vivo* research, *N. sativa* is known as a phytoestrogen [26]. *N. sativa* seeds contain active essential oil compounds, such as TQ, Dithymoquinone, Thymohydroquinone, Thymol, and alpha hederin ( $\alpha$ -hederin). TQ, a component of *N. sativa* which is



predicted to be responsible for estrogenic effects, with ER receptors. *N. sativa* extract at a concentration of 200 µg/ml increased the proliferation of CHO-K1 cells and could reduce the expression of Cyclin E protein in CHO-K1 cells. TQ as a model that represents one of the compounds contained in *N. sativa* extract can bind to ER receptors molecularly.

It can be concluded that the administration of TQ-rich *N. sativa* seed extract has anti-proliferative and pro-apoptotic effects on carcinoma cells, but is protective against healthy cells in the ovaries and other organs.

## Conclusion

In this study, it was found that there is a trend that *N. sativa* is beneficial in protecting the ovaries from damage caused by CIS.

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