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RESEARCH ARTICLE

# The Effect of *Ferula communis* L. on Body-Relative Organ Weight, Serum and Tissue Oxidative Status, Biochemical and Pathological Changes in Rats Exposed to Continuous Light

Buket BOĞA KURU [1 (*)] Mustafa MAKAV [2] Gülden YILDIZ [3] Mükremin ÖLMEZ [4] Mushap KURU [5] Fikret BEKTAŞOĞLU [1] Hatice BEŞEREN [3] Tarkan ŞAHİN [4] Turgut KIRMIZIBAYRAK [1]

[1] Kafkas University, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, TR-36100 Kars - TÜRKİYE
[2] Kafkas University, Faculty of Veterinary Medicine, Department of Physiology, TR-36100 Kars - TÜRKİYE
[3] Kafkas University, Faculty of Medicine, Department of Pathology, TR-36100 Kars - TÜRKİYE
[4] Kafkas University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, TR-36100 Kars - TÜRKİYE
[5] Kafkas University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, TR-36100 Kars - TÜRKİYE

**Abstract**

The goal of this study was to investigate the effects of *Ferula communis* L. on growth performance, relative organ weights, tissue and serum oxidative status, and biochemical and histopathological changes in rats exposed to continuous light. Rats exposed to continuous light for 10 days were given *F. communis* orally. Daily body weight was recorded, and rats were euthanized by cervical dislocation. Blood was collected to determine relative organ weights, followed by immediate biochemical and histopathological analysis of the organs. Serum and tissue oxidative status were measured. Continuous light exposure in rats resulted in weight loss, decreased ovary, uterus, and kidney weights, diminished total antioxidant status (TAS) along with increased cortisol, total oxidant status, and oxidative stress index. However, *F. communis* treatment reduced serum cortisol concentration and alleviated oxidative stress by increasing TAS even under prolonged light exposure conditions. Neither continuous light exposure nor F. communis treatment caused significant changes in malondialdehyde and glutathione in organ tissues. Although continuous light caused an increase in the number of cystic follicles, *F. communis* treatment did not seem to have a positive effect on cystic follicle formation. In conclusion, continuous light exposure stressed the rats and increased cortisol, as well as stimulated oxidative stress and cystic follicle formation. *F. communis* treatment can help alleviate the harmful effects of constant light exposure.

**Keywords:** Body weight, Continuous light stress, Cortisol, Cystic follicle, *Ferula*, Ovary, Oxidative stress

**(*) Corresponding author:** Buket BOĞA KURU
Phone: +90 474 242 6807-5115
Cellular phone: +90 541 513 0609
Fax: +90 474 242 6853
E-mail: buket_vetfak@hotmail.com

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## INTRODUCTION

*Ferula* includes more than 170 genera, with the majority of them being found in Central Asia, the Mediterranean, and North Africa. In order to ascertain their pharmacological characteristics, *Ferula* spp. have been employed in both previous and contemporary studies on humans and animals [1]. In Türkiye, the genus *Ferula* is known as "Çakşır" and is represented by 25 species. The root components are renowned as potent aphrodisiacs in eastern Türkiye [2].

In the genus *Ferula*, components such as coumarin (ferulenol, galbanic acid, and umbelliprenin), coumarin esters (ferulon A, B), sesquiterpenes (germakrans, himacalans, carotenes, humulans, guaians, daucane esters farnesiferol A and B) and sesquiterpene lactones, monoterpene (α-pinene, β-pinene), monoterpene coumarins, prenylated coumarins, sulfur-containing derivatives, phytoestrogens (ferutinin), flavonoids, carbohydrates (galactose, glucuronic acid, arabinose, rhamnose) are well defined. This genus is utilized in traditional medicine for skin infections, psychiatric conditions, high cholesterol, diabetes, arteriosclerosis, gastrointestinal problems, osteo-

porosis, rheumatism, human immunodeficiency virus, type A influenza, cancers (uterine cancer), muscle relaxants, headache, hypertension, toothaches, and vertigo because of its abundant chemical content [2-4].

Studies to identify the phytochemical characteristics of *Ferula* have increased over the last 20 years [3,5-8]. The antioxidant qualities of extracts derived from *Ferula* species have also been the subject of certain studies. *Ferula* extracts are usually thought to have considerable antioxidant activity, according to the research [4,7,9]. *F. communis* extracts have powerful antioxidant capabilities and exhibit no cell toxicity in both traditional *in vitro* assays and *in vitro* cellular models (HL-60). It also possesses intracellular and extracellular scavenging properties. The presence of considerable amounts of phenolic compounds and the interesting and powerful antioxidant capabilities of *F. communis* were also discovered [9].

One of the most significant environmental factors influencing animal behavior is light. The temporal variation of light alters numerous physiological processes in various animal species. The major circadian oscillator in animals is located in the hypothalamic suprachiasmatic nucleus (SCN), and the retinohypothalamic pathway directly links ambient light to SCN neuronal activity. The SCN controls circadian sleep patterns, body temperature, mobility, and nutrition, and these functions may be interfered with when the SCN is negatively impacted by light. Circadian rhythms are synchronized by the length of light and darkness during the day. Circadian rhythm patterns are greatly impacted by variations in the length of the light-dark period. In primates, transitory sleep patterns and body temperature are variably influenced by ambient light, and the SCN controls these circadian cycles [10,11].

Problems including jet lag disorder, delayed sleep phase disorder, and irregular sleep-wake rhythm are experienced as a result of variations in the 24-h circadian cycle's light/dark cycles or sleep-wake cycles [10,12]. These issues can be regarded as severe stressors because they alter the homeostasis of various physiological systems. Stress is brought on by the disruption of sleep and circadian rhythms. Since lighting conditions have a negative impact on sleep, they also have a big impact on stress [13,14]. Distressing lighting alters the physiological and behavioral responses of rats as well as increasing corticosterone levels [15]. Continuous light increases both stress and fear responses in chickens [16].

Animal behavior is orchestrated by a complex interplay of factors. In rodents such as rats, these factors include the estrous cycle in females, exposure to a novel environment, and light [17]. Continuous light exposure has been shown to impact the endocrine system in rats. Specifically, it can increase serum anti-mullerian hormone and potentially exert a stress effect on the endocrine system, as indicated by elevated corticosterone concentration [18]. Moreover, continuous light exposure can increase serum testosterone, luteinizing hormone to follicle-stimulating hormone (LH/FSH) ratio, and the number of cystic-like follicles [19]. Continuous light exposure also caused reproductive activity, endocrine system disorders, and metabolic problems (glucose intolerance) in rats. Similarly, ongoing exposure to light alters the gut flora and brings on reproductive problems like polycystic ovarian syndrome (PCOS) [18].

The objective of this study was to ascertain how *Ferula communis* L. affected the body weight and relative organ weights, tissue and serum oxidative status, and biochemical and pathological alterations in rats exposed to continuous light.

## Material and Methods

### Ethical Approval

The Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2021-153), Kars, Türkiye, gave its clearance before this study could be conducted. According to the *Laboratory Animal Care and Use Manual*, all procedures were carried out.

### Animal

Thirty-two, 3-month-old female Wistar albino rats weighing 275-300 g were utilized. The groups were housed in standard conditions at 23±1°C and 50±5% humidity after a fifteen-day adaptation period. Commercial pellet feed (Bayramoğlu Yem®, Erzurum, Türkiye) and water were given *ad libitum* to the groups.

### Vaginal Cytology

Vaginal cytology was performed on the rats to determine their estrous cycle stage prior to the initiation of the experiment. The study started in diestrus because the luteal phase lasts longer than the follicular phase. Swabs were collected for cytology using the vaginal lavage technique as described in the literature [20]. Swabs were examined under a light microscope (Olympus CX23® Binocular Microscope, Olympus, Japan) after being stained with Giemsa (Merck, Darmstadt, Germany). When a combination of a diminished large cell profile, an elevated neutrophil count, tiny and big nucleated epithelial cells, and a very low number of anucleated keratinized cells were observed in cytology, the time period was regarded as diestrus [20]. Rats in diestrus were used in the experiment.

### Progesterone Analysis

Vaginal cytology was used to determine which rats were in diestrus, and blood was taken from the tail vein and centrifuged at 3000 rpm for 10 min to assess the serum

progesterone concentration. Using commercial kits, a Roche Cobas c501® autoanalyzer (Roche Diagnostics, Mannheim, Germany) was used to measure the serum progesterone concentration. Rats were included in the groups if their progesterone concentration was greater than 1 ng/mL.

### *Ferula communis* L. Extraction and GCMS Analysis

The *Ferula communis* L. roots of a plant were gathered from Ağrı Province, located in the Eastern Anatolia Region of Türkiye, in 2021. The plant was verified by Kafkas University Department of Botanical Sciences in Kars, Türkiye. The root oil was extracted using hydro-distillation with a Clevenger-type instrument for 4 h, using 10 mL of n-Hexane (Merck, Darmstadt, Germany) as the collecting solvent. The oil was then dried with anhydrous sodium sulfate, filtered, and stored in sealed vials at 4°C, protected from light until it was analyzed.

Three samples of oil were obtained through hydro-distillation, and analysis was carried out with GCMS using a capillary column of HP-5MS at 70eV (J & W Scientific, Folsom). The instrument was set up using the procedure developed by Nguir et al.[21]. Sigma-Aldrich, Milan, Italy, provided the standards for the component analysis. Authentic standards provided by the supplier (Sigma-Aldrich, Milan, Italy) and computer matching with the WILEY275, NIST05, and ADAMS libraries, as well as comparisons of mass spectra and retention indices (RI) to those described in the literature [22,23], were used to identify the volatile components. The percentage compositions of the oil components were calculated using electronic integration with flame ionization detection (FID, 280°C) by dividing the area of each component by the sum of the areas of all components separated under these conditions. The percentage values for volatile components were calculated using the average of three oil sample injections. The active compounds of *Ferula communis* L. roots are given in *Table 1*.

### Experimental Design

The groups were formed to be balanced in terms of body weight. Pellet feeds were left in the cage to avoid casting shadows.

The experiment consisted of four groups of rats. The first group (G1) served as the control group and was not given any treatment. They were kept in 12/12 light/dark conditions for ten days. The second group (G2) was also kept in 12/12 light/dark conditions for ten days but was given *Ferula communis* L. orally by gavage at a daily dose of 150 mg/kg. The third group (G3) was kept in 12/12 light/light conditions for ten days with no treatment. The fourth group (G4) was also kept in 12/12 light/light conditions for ten days but was given *Ferula communis* L. orally by gavage at a daily dose of 150 mg/kg. Each group consisted of eight rats.

### Body Weight

Rats were weighed daily for ten days.

### Euthanasia

After intramuscular injections of xylazine HCl (15 mg/kg, Rompun®, Bayer, Germany) and ketamine HCl (75 mg/kg, Ketasol®, Richter Pharma AG, Austria), rats were euthanized by cervical dislocation at the end of the study. After that, rapid blood collection and organ sampling were performed.

### Blood Collection

A gel-vacuum tube (BD Vacutainer® SST II Advance, Becton, Dickinson and Company, UK) was used to collect blood through a cardiac puncture. The blood was centrifuged (NF 400R®, Nüve, Türkiye) at +4°C for 10 min at 3000 rpm. Following centrifugation, the sera were separated into individual microcentrifuge tubes and kept at -18°C until biochemical analyses were performed.

***Table 1.*** *The chemical composition of the essential oil from Ferula communis L. roots.*

| No | Compounds | % Composition | No | Compounds | % Composition |
|---|---|---|---|---|---|
| 1 | Dillapiole | 7.9 | 11 | (E)-nerolidol acetate | 3.4 |
| 2 | Guaiol | 7.3 | 12 | (Z)-lanceol | 3.1 |
| 3 | Spathulenol | 6.8 | 13 | β-Barbatene | 6.1 |
| 4 | Myristicin | 6.0 | 14 | Epizonarene | 6.1 |
| 5 | T-cadinol | 5.9 | 15 | α-Zingibirene | 4.7 |
| 6 | β-Gurjunene | 3.4 | 16 | β-Bisabolene | 5.1 |
| 7 | (E)-β-farnesene | 4.7 | 17 | γ-Cadinene | 4.8 |
| 8 | (E)-nerolidol | 3.5 | 18 | Trans- Calamenene | 3.9 |
| 9 | T-Muurolol | 3.0 | 19 | Elemicin | 6.0 |
| 10 | α -Cadinol | 2.8 | - | - | - |

### Organ Weights

The stomach, liver, kidneys, spleen, total intestine, uterus, and ovaries were dissected immediately after euthanasia. The thorax was opened, and the heart and lungs were taken out and weighed. The eyes were also taken out and weighed. The aforementioned organs were removed from their surrounding tissues, and they were then instantly weighed to three decimal places (dual organs weighed together). The organs were divided for biochemical and histological analysis just after weighting. Separated for biochemical analysis were the right ovary, kidney, and eye, while for histopathological evaluation were the left ovary, kidney, and eye.

### Biochemical Analyses

A fully automated ADVIA Centaur® XP analyzer (Siemens Healthcare Diagnostics, Tokyo, Japan) and a compatible commercial kit were used to assess serum 25-OH-vitamin D (vit D) and cortisol concentrations using the chemiluminescent immunoassay method.

Serum glucose, calcium (Ca), magnesium (Mg), and phosphorus (P) were measured by the photometric method using a fully automatic Abbot® ARCHITECT analyzer (Abbott Diagnostics, Lake Forest, IL, USA) and commercial kits.

Total antioxidant status (TAS) and total oxidant status (TOS) in serum were measured using a commercial kit (Rel Assay Diagnostics®, Mega Tıp, Türkiye) using the colorimetric method.

The oxidative stress index (OSI) was initially defined as the ratio of TOS to TAS. The resultant unit of TAS, mmol Trolox equivalent/L, was changed to µmol Trolox equivalent/L in order to do the calculation, and OSI was then determined using the formula below [24]:

OSI (Arbitrary unit) = TOS (µmol $H_2O_2$ Eq/L)/TAS (µmol Trolox Eq/L) x 100

For malondialdehyde (MDA) and glutathione (GSH) analyses, organ samples from the heart, kidney, uterus, ovary, and eye were homogenized with phosphate buffer (pH 7.4), and their homogenates were separated by centrifugation at 3000 rpm for 10 min. Before performing MDA and GSH measurements, homogenates were placed into separate microcentrifuge tubes and kept at -18°C. GSH and MDA analyses were performed on tissue homogenates using the methods described by Beutler et al.[25] and Yoshoiko et al.[26], respectively.

### Histopathology

The tissues were fixed for eight hours in 10% buffered formaldehyde. Tissues were embedded in paraffin after a typical histological follow-up. Hematoxylin & Eosin was used to stain the tissues after they had been cut into 4 µm pieces and deparaffinized in an oven for 40 min at 62°C. Lastly, histopathologic features were assessed by light microscopy (Olympus BX46®, Olympus Corporation, Japan).

The left ovary sections were examined for the presence of Graafian follicles [27,28], cystic follicles, and corpus luteum [18,28].

### Statistical Analysis

Before commencing the study, the sample size was determined through a power analysis using G*Power version 3.1.9.7 (University of Düsseldorf, Germany). The calculation was based on a test power of 0.95, a significance level of 0.05, and an effect size (d) of 1.83, as reported in a study [29].

The Shapiro-Wilk test was used to evaluate the normal distribution of the data. A one-way analysis of variance (ANOVA) was performed to compare normally distributed data. Following the check for homogeneity of variances with Levene's test, the Tukey honestly significant difference (HSD) test was used with the post-hoc comparison. When the variables were not normally distributed, Kruskal-Wallis ranked one-way analysis of variance was used for multiple comparisons, and pairwise comparisons of parameters with significance were evaluated using the Mann-Whitney U test. A Bonferroni correction was applied to the P value in this test result (adjustment significant P value). The chi-square test or Fisher's exact test was used to compare the ratios of Graafian follicles, cystic follicles, and corpora lutea in the groups. Data are given as mean ± standard deviation (SD). GraphPad Prism® (Version 8.0, GraphPad Software Inc., San Diego, CA, USA) and SPSS® (Version 26.0, SPSS Inc./IBM Group, Chicago, IL, USA) programs were used for statistical analysis. A P value of $<0.05$ was considered significant in the multiple or pairwise comparisons.

## Results

### Body Weight Changes

In terms of body weight change over time, there was no statistically significant difference among the groups ($P>0.05$). The control group's body weight did not alter noticeably (G1). Rats lost weight when exposed to constant light (G3). Gaining body weight was positively impacted by *F. communis* (G2). Continuous light's detrimental impact on body weight was not identified in G4. *Fig. 1* displays the change in body weight in rats.

### Organ Weights

The effects of continuous light exposure on the weight of the liver, spleen, stomach, whole intestine, heart, lungs,

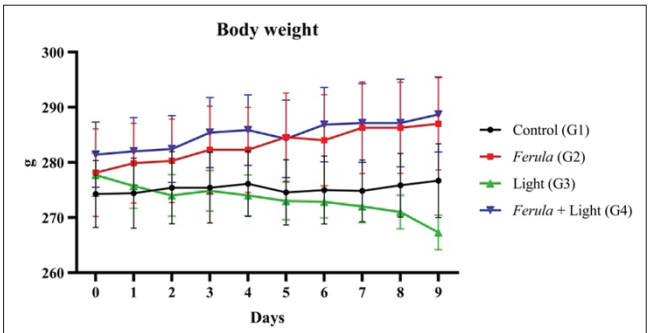


**Fig 1.** The differences in body weight among the groups. Across the groups, there was no statistically significant difference in body weight (P>0.05). Rats' body weight was affected negatively by continuous light but positively by *Ferula communis*

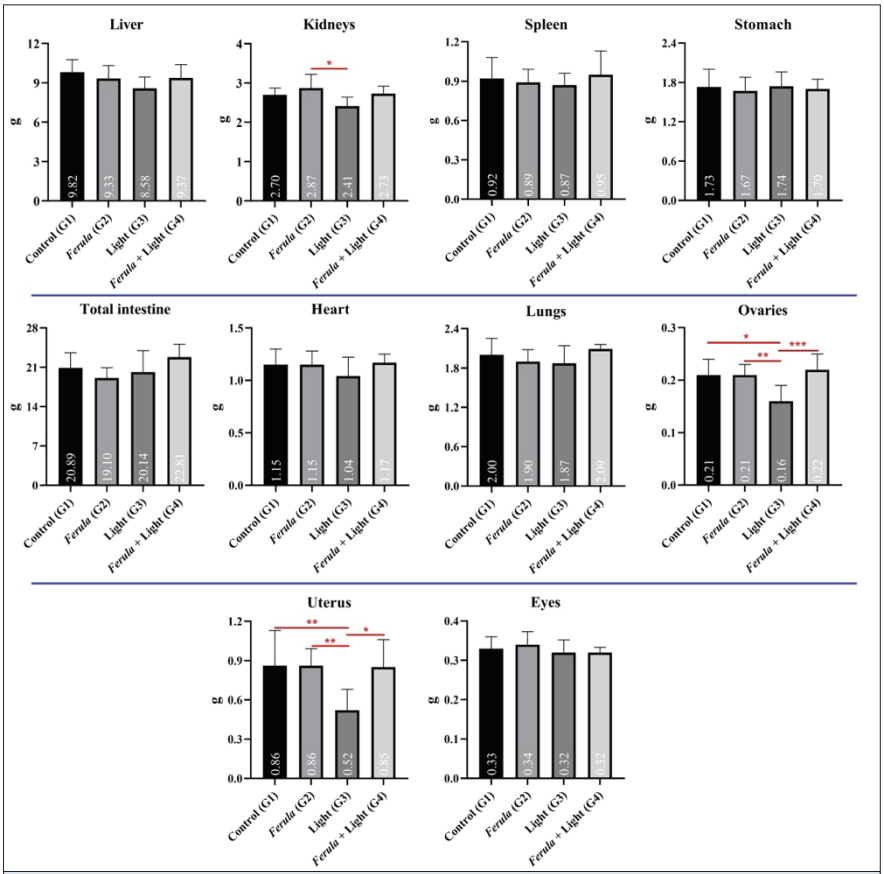


**Fig 2.** The changes in relative organ weights depending on the group. Continuous light exposure or *Ferula communis* treatment had no effect on the weight of the liver, spleen, stomach, total intestine, heart, lungs, or eyes (P>0.05). Kidney weight increased with *F. communis* treatment (G2) and decreased with continuous light exposure (G3). After exposure to light for an extended period of time, the ovary and uterus lost weight. Continuous light exposure had a deleterious impact on ovarian and uterine weight; however, this was mitigated by *F. communis* therapy. *: P<0.05, **: P<0.01, ***: P<0.001

and eyes were not statistically significant (P>0.05). The relative weights of the organs were unaffected by the *F. communis* treatment, either positively or negatively *(Fig. 2)*. The relative kidney weight was statistically different between the G2 and G3 groups, increasing in G2 but decreasing in G3 (P=0.017, *Fig. 2)*.

Regarding relative organ weight, prolonged exposure to light had the greatest impact on the uterus and ovaries *(Fig. 2)*. A statistical significance was found in the multiple comparisons of ovarian weight between groups (P=0.003). In G3 compared to G1, G2, and G4, ovarian weight was significantly decreased (P=0.023, P=0.01, and P=0.005, respectively). Relative uterine weight was changed by continuous light exposure, and multiple comparisons of groups were statistically significant (P=0.009). Uterine weight was lower in G3 than in G1 (P=0.02), G2 (P=0.02),

and G4 (P=0.025). Surprisingly, *F. communis* treatment (G4) seems to have removed the deleterious effects of continuous light exposure on both ovarian and uterine weight *(Fig. 2)*.

## Biochemical Changes

The highest serum vit D concentration was measured in G3, there were no statistically significant differences among groups. (P>0.05, *Fig. 3)*.

Continuous light exposure in G3 generated a substantial increase in serum cortisol concentration compared to the other groups (G1, G2, and G4). Multiple comparisons for serum cortisol concentration were statistically significant (P=0.003). *F. communis* tends to lower serum cortisol concentrations even with prolonged exposure to light (G4). Surprisingly and remarkably, there was a statistical difference (P=0.04) in blood cortisol between G3 and

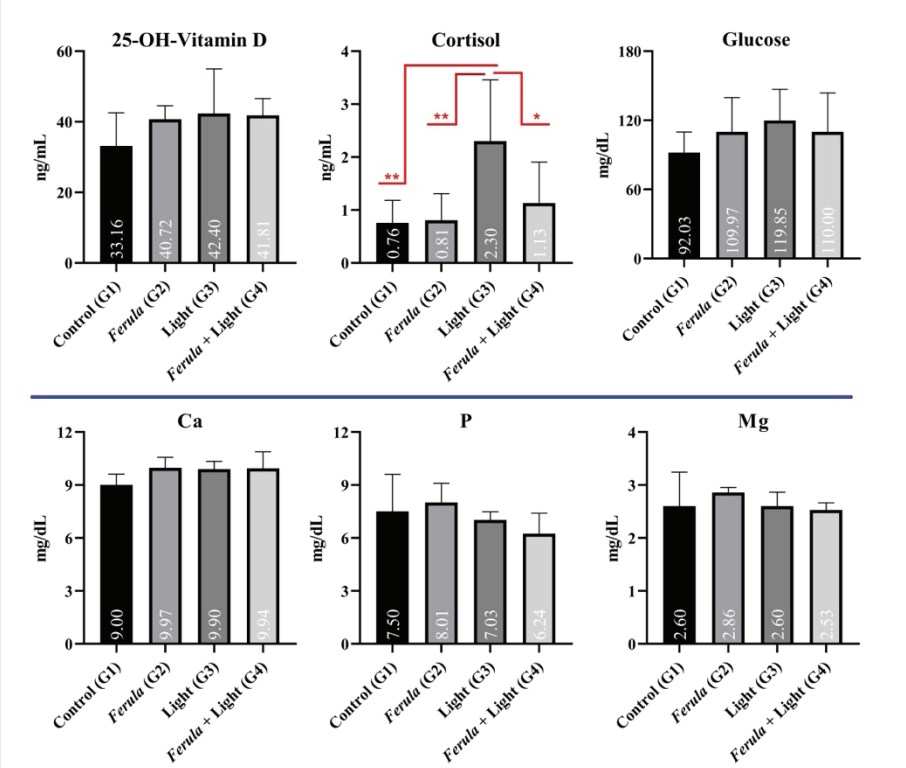


**Fig 3.** Group-based variations in several biochemical parameters. The difference in blood cortisol concentrations between G3 and G4 is crucial. Vitamin D, glucose, Ca, Mg, and P were unaffected by either continuous light exposure or *Ferula communis* treatment. *: P<0.05, **: P<0.01

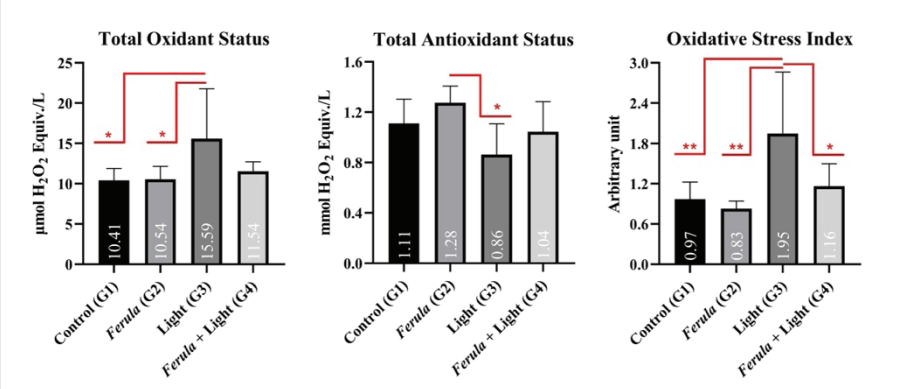


**Fig 4.** Change of oxidative status in groups. Continuous light exposure (G3) increased total oxidant status (TOS) and oxidative stress index (OSI) and decreased total antioxidant status (TAS). *Ferula communis* treatment, on the other hand, decreased TOS and increased TAS when exposed to continuous light, but these effects were limited. But when exposed to constant light, the *F. communis* treatment reduced OSI. OSI was statistically lower in G4 than in G3, suggesting that *F. communis* may be effective in reducing oxidative stress. *: P<0.05, **: P<0.0

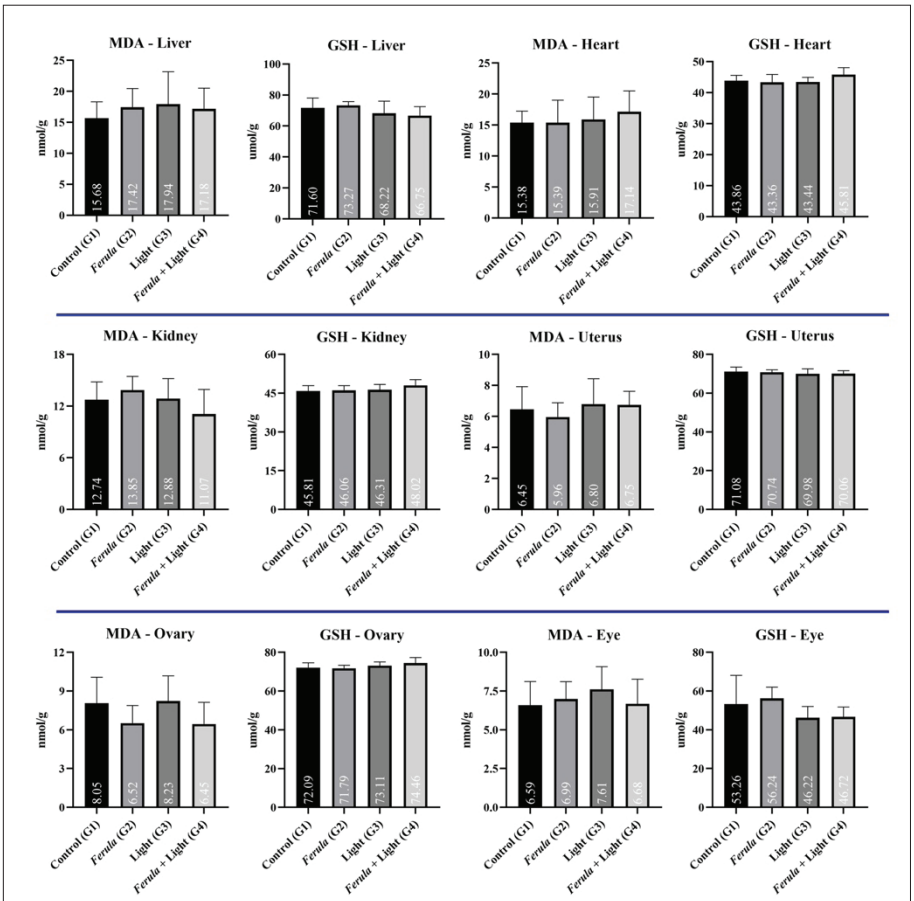


**Fig 5.** Change of tissue oxidative status in groups. Malondialdehyde (MDA) and glutathione (GSH) in the liver, heart, kidney, uterus, ovary, and eye were not significantly changed by continuous light exposure or treatment with *Ferula communis* (P>0.05)

G4 *(Fig. 3)*. Again, serum cortisol concentration was not altered in G4 exposed to continuous light. The absence of a significant difference between G1 and G4 shows that *F. communis* is able to avoid an increase in cortisol following continuous light exposure *(Fig. 3)*.

Serum glucose concentrations were not statistically significant among groups (P>0.05). The variation in serum glucose concentration according to groups is shown in *Fig. 3*. Continuous light exposure or *F. communis* treatment did not have a statistically significant effect on serum Ca, P, and Mg concentrations (P>0.05, *Fig. 3)*.

## Serum Oxidative Status

There was a statistically significant difference in TOS between the groups (P=0.025). Constant light exposure enhanced TOS in rats. The increase in TOS in G3 was significantly different compared to G1 (P=0.037) and G2 (P=0.043) *(Fig. 4)*. The *F. communis* treatment (G4) reduced continuous light stress but was not statistically significant compared to G3 (P>0.05).

Although the *F. communis* therapy (G2) increased TAS compared to G1 and G4, it was not statistically significant. TAS decreased as a result of continuous light exposure (G3). Therefore, there was a statistically significant difference between G2 and G3 (P=0.005). Although there was no statistically significant difference, TAS appears to be higher in G4 than in G3 *(Fig. 4)*.

Continuous light exposure (G3) increased OSI in rats. OSI in G3 was statistically higher than in G1 (P=0.007), G2 (P=0.002) and G4 (P=0.04) *(Fig. 4)*. The *F. communis* treatment had a positive effect on the reduction of OSI. OSI was lower than G3 in G4 and statistically similar to G1 and G3 *(Fig. 4)*.

## Tissue Oxidative Status

Rat liver, heart, kidney, uterus, ovary, and eye MDA and GSH levels were not significantly affected by continuous light exposure or *F. communis* treatment (P>0.05, *Fig. 5)*. Thus, continuous light stress or treatment with *F. communis* had no effect on the tissue's oxidative status *(Fig. 5)*.

## Histopathology

The livers of all rats did not exhibit any signs of necrosis, apoptosis, or fibrosis. There was only mild

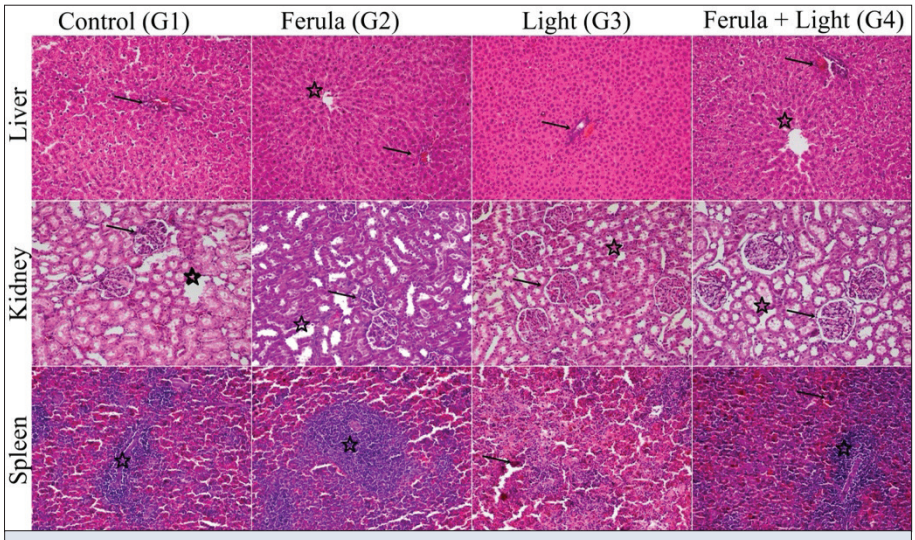


**Fig 6.** Histopathological changes in various organs (H&E x200). Liver - G1: Liver tissue of the control group (H&E, x200) in the usual histomorphology with the portal area indicated by the arrow. G2: Portal area shown with an arrow, central vein with an asterisk. Liver tissue of the *Ferula communis* group in usual histomorphology (H&E, x200). G3: The liver tissue of the light group with mild portal inflammation, the portal area is indicated by the arrow (H&E, x200). G4: Mild portal inflammation with portal area with arrow and central vein with asterisk, liver tissue of the Light + *F. communis* group (H&E, x200). Kidney - G1, G2, G3, and G4: Arrows represent glomeruli, stars represent tubules. Renal tissue samples in usual histomorphology (H&E, x200). Spleen - G1, G2: Samples of spleen tissue in usual histomorphology. Asterisks indicates white pulp (H&E, x200). G3: The arrow indicates one of the hemosiderin-loaded macrophages in the red pulp. Pigmentation of the macrophage represents the sign of bleeding (H&E, x200). G4: A star indicates white pulp, and an arrow indicates one of the hemosiderin-loaded macrophages in the red pulp. Pigmentation of the macrophage represents the sign of bleeding (H&E, x200)

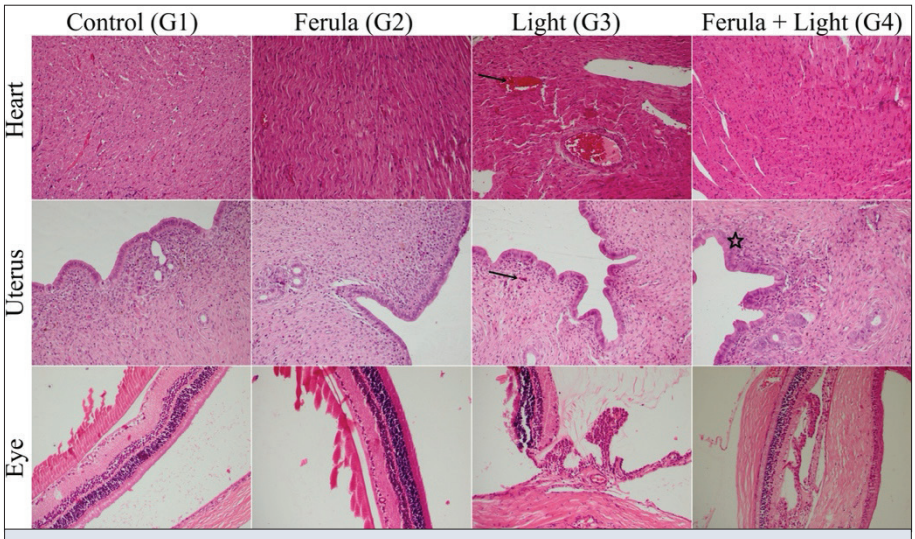


**Fig 7.** Histopathological changes in various organs (H&E x200). Heart - G1, G2, G3, and G4: Striated muscle cells in sections of the heart. G3: Slight congestion is observed in the vessels in the sections belonging to the light group with the arrow sign (H&E, x200). Uterus - G1, G2: Normal histomorphology shows endometrial glands, stroma, and myometrium in sections (H&E, x200). G3: One of the hemosiderin-loaded macrophages representing the bleeding sign in the light group is shown with an arrow (H&E, x200). G4: Signs of endometritis are observed in the focal area, with a few neutrophil infiltrations marked with an asterisk (H&E, x200). Eye - G1, G2, G3, G3: Retinal tissue samples in usual histomorphology (H&E, x200)

portal inflammation and congestion, and no observable difference in morphology between the groups. The kidneys had minimal congestion, hyaline cast deposition in some areas, and calcification in collecting ducts in a few tissues, but no evidence of necrosis, apoptosis, or inflammation. The spleen tissue of G1, G2, and G4 had areas of congestion and hemosiderin-loaded macrophages, but no other pathological findings. G3 showed more prominent bleeding and congestion, but not significantly different from the other groups *(Fig. 6)*. The heart tissues showed

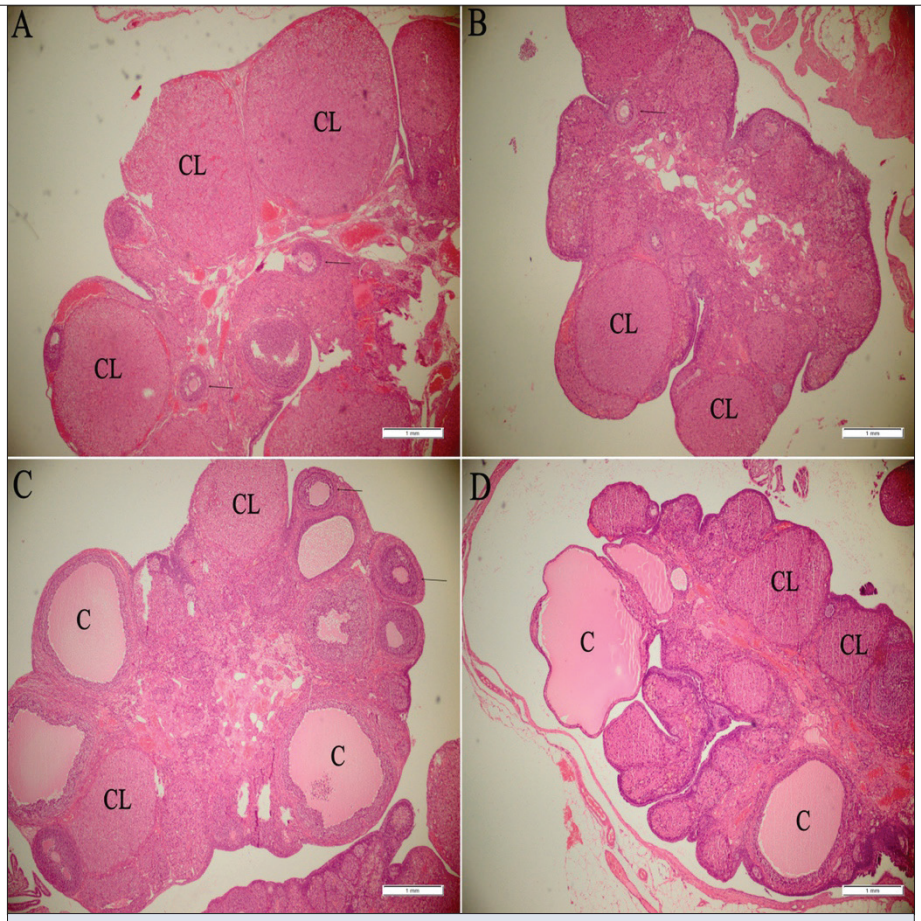


**Fig 8.** Ovarian histopathological alterations in the various groups. **A:** Numerous secondary follicles *(arrow)* and corpus luteum (CL) are seen in the G1 (H&E, x40), **B:** In G2, the corpus luteum (CL) and the primary follicle, which is marked by an arrow, are visible (H&E, x40), **C:** In G3, there are more secondary follicles *(arrow),* corpus luteum (CL), and follicular cysts (C) (H&E, x40), **D:** A considerably smaller corpus luteum (CL) and enlarged follicular cysts (C) in G4 (H&E, x40)

bleeding and congestion, but no other pathological finding. Uterine samples were examined for congestion, hemorrhage, endometritis, and hyperplasia, but none of the rats had hyperplasia. Some rats had focal endometritis and hemosiderin-loaded macrophages, indicating previous bleeding. Ocular tissue samples consistently showed the typical histomorphological appearance. The typical histomorphological appearance was consistently seen in the ocular tissue samples *(Fig. 7)*.

The ovary was examined in different groups of rats, and in G1, the structures looked normal, with a corpus luteum present and no cystic follicles seen in the ovarian sections *(Fig. 8-A)*. In G2, two rats lacked corpora lutea, while two others had cystic follicles *(Fig. 8-B)*. In G3, cystic follicles were found in almost all rats and were also present in the corpus luteum *(Fig. 8-C)*. Finally, in G4, all rats had both cystic follicles and the corpus luteum *(Fig. 8-D)*.

There were no significant differences between the groups regarding the presence of Graafian follicles or corpora lutea (P>0.05). However, while no cystic follicles were found in G1, the highest rate of cystic follicles was observed in G3 and G4 (P<0.05). Exposure to continuous light was found to increase the incidence of cystic follicles in rats. Interestingly, in G3 and G4, where the rate of cystic follicles was high, all rats still had corpus luteum. Although continuous light exposure increased the number of cystic follicles, treatment with *F. communis* did not have a positive effect on cystic follicle formation *(Table 2)*.

***Table 2.*** *Ratio of Graafian follicle, cystic follicle and corpus luteum in groups*

| Groups | Graafian Follicles % (+/-) | Cystic Follicles % (+/-) | Corpus Luteum % (+/-) |
|---|---|---|---|
| Control (G1) | 100 (8/0) | 0[a] (0/8) | 100 (8/0) |
| *Ferula* (G2) | 87.5 (7/1) | 25[a] (2/6) | 75 (6/2) |
| Light (G3) | 100 (8/0) | 87.5[b] (7/1) | 100 (8/0) |
| *Ferula* + Light (G4) | 100 (8/0) | 100[b] (8/0) | 100 (8/0) |

[a-b] *Different letters in the same column are statistically significant (P<0.05)*

## Discussion

*Ferula communis* exhibits a wide range of pharmacological effects both *in vitro* and *in vivo*, such as antidiabetic, antibacterial, antiproliferative, and cytotoxic actions. It comprises various bioactive compounds with diverse biological activities [4,21,30]. Likewise, several studies have shown that *Ferula communis* possesses potent antioxidant activity [9,31]. Therefore, our goals were to induce stress in rats by continuously exposing them to light and to evaluate the effects of Ferula communis treatment on body weight, relative organ weight, serum and tissue oxidative status, and biochemical and pathological changes. *F. hermonis* treatment had a positive effect on body weight gain in growing female and male rats [32]. Continuous light exposure has a limited effect on body weight [15,18,33]. Short-term continuous light exposure in our study slightly decreased body weight gain, but it was not statistically effective. However, if the light duration was extended, perhaps it would have an effect on body weight. The decrease in weight gain can be explained by the fact that continuous light exposure reduces feed consumption [34]. In the present study, rats exposed to continuous light exhibited a favorable response to *F. communis* treatment in terms of weight gain.

In our study, neither continuous light exposure nor *F. communis* treatment had a significant effect on most of the relative organ weights in rats, which was further supported by histopathological findings. Continuous light exposure did, however, adversely affect the relative weights of the ovaries and uterus in rats. The G3 group had significantly lower ovary and uterus weights compared to the other groups. Similar results were observed in a study where rats were exposed to continuous light for an extended period [35]. In this study, *F. communis* appeared to mitigate the negative effects of continuous light on ovary and uterus weight. It is possible that continuous light exposure disrupts the estrous cycle [18], leading to lower genital organ weights in G3. *F. communis* may have facilitated more regular estrous cycles through its estrogenic effects. A study supporting our findings reported that *Ferula* can be used as a therapy for female infertility [36]. In our study, kidney weight was not significantly affected by continuous light exposure but was lower in the *F. communis* group compared to the control group. Histopathologically, the kidney tissue was normal in all groups. This was an interesting finding. Perhaps continuous light exposure led to reduced water intake in rats, which in turn could have contributed to the decrease in kidney weight [34].

Long-term exposure to light can interfere with circadian rhythms, which has an impact on key physiological processes and organ systems [37]. Agonistic behaviors that disturb the in-group order are also brought on by constant light exposure in some animals [15]. Along with in-group restlessness, disrupted physiological processes and agonistic behaviors lead to stress, and the most significant consequence of this is an increase in corticosterone concentration [15,38]. As blood cortisol concentration was found to be statistically higher in G3 compared to the other groups, our study suggests that continuous light exposure (G3) induces severe stress. While the behavior of the groups was not directly observed, it is inferred that G3 experiences severe stress due to constant illumination. The results in G4 were also highly intriguing, as *F. communis* may have prevented the increase in serum cortisol concentrations following prolonged exposure to light. G4's serum cortisol concentrations was lower than that of G3 because it was comparable to that of G1 and G2. *F. communis* may include anticatabolic substances that function by inhibiting cortisol receptors, yet it is difficult to describe how *Ferula* carries out this activity [39]. Also, there is evidence to suggest that the ferulic acid found in *F. communis* lowers corticosterone concentrations [40].

*F. communis* possesses a large number of antioxidants and has been proven to be a potent radical scavenger in numerous tests [3,4,30]. The active component of *Ferula*, ferutinin, possesses antioxidant, antibacterial, anti-inflammatory, estrogenic, and anti-cancer properties [41]. *Ferula* contains flavonoids, which act as natural antioxidants and free radical scavengers [42]. Numerous studies have demonstrated that a wide range of diseases and inflammatory conditions trigger and exacerbate oxidative stress in the organism [43-45]. Stress was induced in this study by continuous light exposure, and an increase in serum oxidative stress parameters was also observed. Constant illumination increased TOS and OSI while decreasing TAS in G3. This was able to be changed by the *F. communis* treatment, which increased TAS in G4. Again, OSI decreased more in G4 than in G3. No appreciable variations in tissue oxidative status were discovered. In any event, prolonged exposure to light did not seriously damage the tissues. The ovary was found to be more sensitive to continuous light exposure than other organs in histopathological investigations. The results show that *F. communis* can be employed as a radical scavenger to defend against oxidative stress.

Continuous light exposure can form polycystic ovary-like structures in rats. Continuous light can prevent the formation of the luteinizing hormone peak in rodents, preventing ovulation from triggering. In earlier studies, this topic has been thoroughly examined [18,46,47]. In our study, continuous light induced cystic follicle formation. Cystic follicles were detected in both G3 and G4 groups. The presence of corpus luteum alongside these cystic follicles suggests that cyclic activity continued in the rats. While serum progesterone levels were not measured at

the study's conclusion, histopathological analysis revealed the clear presence of numerous corpus luteum. Despite the estrogenic properties of Ferula, it appears to have partially induced cystic follicle production. However, corpus luteum were not identified in two G2 rats. This may have been brought on by *Ferula*'s estrogenic effects. Moreover, the rate of cystic follicles was not sufficiently decreased with *F. communis* treatment. The percentage of cystic follicles in G4 was statistically similar to G3. The impact of *Ferula* on the cystic follicle may become clearer with continued use. It should be emphasized that cystic follicles are less frequent in G4 histopathologically. In patients with polycystic ovaries, *F. communis* treatment has already decreased the number of cystic follicles [48,49]. *F. communis* may have successfully treated it by suppressing the increased androgen concentration in the polycystic ovary with an estrogenic effect [48].

In conclusion, continuous light exposure can cause stress and increase cortisol concentration in rats. It can also lead to oxidative stress and cystic follicle formation. *Ferula* treatment can reverse the harmful effects of continuous light exposure with its antioxidant and estrogenic effects. There may still be some unknowns regarding *Ferula's* ability to eliminate cystic follicles. Therefore, detailed studies are needed to determine the effects of short-term or long-term use of *F. communis*.

## Declarations

**Availability of Data and Materials:** The data that support the findings of this study are available from the corresponding author (B. Boğa Kuru) upon reasonable request.

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**Ethical Approval:** Kafkas University Local Ethics Committee for Animal Experiments (KAÜ-HADYEK/2021-153), Kars, Türkiye, gave its clearance before this study could be conducted.

**Conflict of Interests:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Author Contributions:** BBK, MM: Designing the study, MM, MK, FB, BBK: Implementation of the experimental protocol, data collection, BBK, TK: Data interpretation, data analysis and statistical analysis, MM, MK: Biochemical analysis, MÖ, TŞ: Extraction and GCMS analysis, GY, HB: Histopathology, BBK: Writing-Original drafting. All authors contributed to the article and gave final approval of the version to be submitted.

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