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# Morphometric and Histological Changes in Ovary and Mammary Glands of Suckling Rats under Effects of High Doses of Rosemary Oil Extract

**Asmaa J. Basheer** **Fatimah Q. Mohammad**
Department of Biology/ College of Science/ University of Mosul/ Mosul/ Iraq

**Al-Kassim N.A. Hasan**
*Department of Physiology/ Biochemistry and Pharmacology/ College of Veterinary Medicine/ University of Mosul/ Mosul/ Iraq*

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**corresponding author:**
**Asmaa Jebur Basheer**
Asmaa20scp49@student.uomosul.edu.iq
**Fatimah Q. M. Al-Hayyali**
fatsbio25@uomosul.edu.iq
**Al.Kassim, N.A.H**
nadhem_1957@yahoo.com

## ABSTRACT

Rosemary *Rosemarinus officinalis* is a medicinal plant that has long been used by people all over the world, but many of its constituents may be harmful to the body. This study looks at the effect of *R. officinalis* oil extract on the ovaries and mammary glands structure of Sprague Dawley female rats. The chemical analysis of the tested essential oil was performed with the help of gas chromatography and mass spectroscopy (GC-MS). Suckling female rats were given orally *R. officinalis* oil extract at doses of 250, 500, and 1000 mg/kg body weight for 21 days, while control rats were given corn oil. Following this, tissue samples were obtained from these organs in order to study histomorphometric and histological changes. The number of primaries, secondary, graffian, and atretic follicles, as well as corpora lutea, were then determined. When compared to the control group, all doses of rosemary oil significantly reduced the number of graffian follicles and corpora lutea in the ovary. Furthermore, the number of atretic follicles significantly increased. Different doses of rosemary oil extract were used appeared congestion, collagen fiber deposition, ovarian atrophy, vascular degeneration, coagulative necrosis, oedema, degenerated lactating alveoli, and fatty replacement phenomenon in the ovary and mammary gland tissue. The current study demonstrates that rosemary oil effects on the ovary and mammary glands of female rat can cause significant structural changes, these changes increased with increasing doses of rosemary oil, this can have an impact on the reproductive function and fertility of exposed animals, as a result, the use of rosemary oil in pediatrics and pregnant or suckling women should be approached with caution due to the toxicity.

**Keywords:** Rosemary oil, ovary, mammary gland, histopathological changes, morphometric.

## INTRODUCTION

Nature is an important source of bioactive molecules with potential utility in many diseases; herbal drugs were used to treat a broad spectrum of diseases and/or to modify non-pathological states (Abu-Al-Basal, 2010). The secondary metabolites of plants are known to have therapeutic effects; many have been used to treat different diseases, such as brain (Seo *et al*., 2018), obesity (De Freitas *et al*., 2013), skin diseases (Ariffin and Hasham, 2016) and Liver and kidney diseases (Mohammed *et al*., 2022).

*Rosmarinus officinalis* L., known as rosemary, is an evergreen shrub native to the Mediterranean region which belongs to the Lamiaceae family. In a recent phylogenetic analysis, the genus Rosmarinus has been merged into the genus Salvia. This means that the *Rosmarinus officinalis* is no longer the correct name of the species studied. Since the name *Salvia officinalis* was already occupied when the merger was done, this species needed a new specific epithet in Salvia, so it is now known under the name *Salvia Rosmarinus* (UniProt, 2020). It is an aromatic plant with needle-like leaves that is cultivated worldwide. Its leaves have been extensively used as seasoning as well as in traditional medicine for treating several disorders such as renal colic and respiratory diseases (Tian *et al*., 2010). This plant has therapeutic properties and has been used in folk medicine as an oral preparation to relieve renal colic, dysmenorrhea, and muscle spasms (Ribeiro-Santos *et al*., 2015). Rosemary also has antifungal, antiviral, and antibacterial activities (Soulaimani *et al*., 2021; Sales and Pashazadeh, 2020), anti-inflammatory, antitumor, antithrombotic, anti-nociceptive, antidepressant, antioxidant, anti-proliferative activities (Bouyahya *et al*., 2020; Nguyen *et al*., 2021; Ielciu *et al*., 2022). Several medicinal applications for this plant have been identified, such as treatment of disorders associated with the nervous, cardiovascular, gastrointestinal, menstrual, hepatic, and reproductive systems and with respiratory and skin conditions (Begum *et al*., 2013). Owing to its diverse properties, rosemary has also been used widely in the food and cosmetics industries (González-Minero *et al*., 2020).

The biological activities of secondary metabolites and extracts of *R. officinalis* were reported in many studies investigating various effects such as its antitumor, antioxidant, anti-infectious, anti-inflammatory, and analgesic activities and effects on the central nervous system, endocrine system, body weight changes, cerebral ischemia, hepato-nephrotoxicity, stress, and anxiety (Andrade *et al*., 2018; De Oliveira *et al*., 2019). The presence of carnosol and carnosic, rosmarinic, ursolic, oleanolic, and micromeric acids, which act synergistically, has been attributed to rosemary's anti-inflammatory activity (Altinier *et al*., 2007; Lee *et al*., 2017). Topical treatments containing rosemary oil may be hazardous to hypersensitive people who are allergic to camphor. However, rosemary oil has been linked to a number of negative side effects. Lemonica *et al*., (1996) discovered an anti-implantation effect of it and an aqueous extract of rosemary leaves in Wistar rats. Rosemary oil, when taken orally, can cause convulsions; epileptic patients should use this oil with caution and never consume quantities greater than those found in foods, especially during pregnancy.

Given the widespread use of rosemary essential oil, reports of some negative side effects, and a lack of toxic evaluations of this oil in female reproductive analysis in experimental rats, therefore this study came to investigate the several doses of volatile rosemary oil extract effects on the morphometric and histotoxic of ovary and mammary gland in suckling rats.

## MATERIALS AND METHODS

### Preparation of essential oil extract

Rosemary plants were collected from the gardens of Mosul university and then washed and dried in dark conditions at room temperature. The dried leaves were immersed in 1 liter of sterile distilled water in a spherical glass flask using a Clevenger device. Then distillation was carried out for 4 hours and after the completion of the process the system was left to cool, followed by collecting the oil and getting rid of the remaining water with the oil using anhydrous sodium sulphate and keeping the oil in an opaque bottle at a temperature of 4°C (Jalali-Heravi *et al*., 2011).

**Phytochemical analysis by GC/MS**

Gas chromatography-Mas spectrometry (GC-MS) analysis of Rosemary essential oil was performed in the scientific center at Soran university/ Erbil/ Iraq by using a GC-MS model-Agilent 7890B with colum type: J and WDB-5ms ultra inert GC column-30m-0.25mm-0.25µm. Helium gas was used as a carrier gas at a flow rate of (1ml/min) (Jalali-Heravi *et al*., 2011).

**Animal's model**

24 female albino pregnant rats have been used at age of 10-12 weeks and their weights 197.35± 7.32 g. They have been obtained from animal house in the college of Veterinary Medicine/ University of Mosul. The rats were housed in plastic cages (6 rats per cage) with stain less steel cover with dimensions 15*15*30 cm. Rats were kept at standard conditions (temperature was 20-25°C, and 12h light/dark cycle) and feeding add libtum.

**Experimental protocol**

Twenty-four pregnant female rats were divided into four subgroups. Each group included 6 rats. All treatments were given orally daily by rosemary oil for 21 days (suckling period) as group one group1 served as the control and given corn oil, other three groups received oral rosemary oil via stomach tubes at daily doses of 250, 500 and 1000 mg/kg respectively.

**Morphometric analysis**

They used the SPSS software version 22 to analyses the ovarian follicles using one-way analysis of variance: ANOVA and the Tukey posthoc test (IBM Corp., Armonk, NY, USA). A statistically significant p-value of 0.05 was used. The data was presented in the form of mean standard error of the mean (SEM).

**Histopathological preparation**

Internal organs, ovary and mammary glands were removed. Then they were placed in solution of 10% formalin for a period of 24 hours for fixation, after that the tissues were dehydrated using gradual concatenations of alcohol solution 50% -100% for 5 minutes each. Then the tissue samples were cleared in 2 separated xylene changes prior to placing them in paraffin wax for final sectioning. Later the samples were sectioned at 5µm thicknesses, then it stained by hematoxylin Harris and eosin stain to study the histological changes compared to the control group using light microscope (Survarna *et al*., 2013).

## RESULTS

After obtaining the volatile oil extract of rosemary and then analyzing its active compounds, as the gas chromatography technique showed that the rosemary oil extract consists of many active compounds, the major important of these compounds are 1,8-cineole 48.49% , alpha-pinene 12.57%, camphor 10.24%, beta-pinene 8.22, Camphene 5.44, Cymene 4.33, Borneol L 3.90, α-terpineol 1.44%, beta-Myrcene 1.28%, l-Bornyl acetate 1.08%, and other components listed in (Table 1).

**Table 1: phytochemical compounds detected in essential oil of rosemary oil via GC/MS.**

| No | RT (Min) | Area% | Name | Quality | CAS Number |
|---|---|---|---|---|---|
| 1 | 6 | 0.06 | Tricyclene | 96 | 000508-32-7 |
| 2 | 6.157 | 0.11 | A-Thujene | 97 | 002867-05-2 |
| 3 | 6.409 | 12.57 | Alpha. -Pinene | 96 | 000080-56-8 |
| 4 | 6.823 | 0.73 | Alpha. -Fenchene | 97 | 000471-84-1 |
| 5 | 6.886 | 5.44 | Camphene | 98 | 000079-92-5 |
| 6 | 7.903 | 8.22 | Beta. -Pinene | 97 | 018172-67-3 |
| 7 | 8.453 | 1.28 | Beta. -Myrcene | 96 | 000123-35-3 |
| 8 | 8.941 | 0.28 | L-Phellandrene | 95 | 000099-83-2 |
| 9 | 9.449 | 0.07 | Alpha. -Terpinene | 98 | 000099-86-5 |
| 10 | 9.837 | 4.33 | Cymene | 97 | 000527-84-4 |
| 11 | 10.188 | 48.49 | 1,8-Cineole | 98 | 000470-82-6 |
| 12 | 10.393 | 0.04 | Trans-. Alpha. -Ocimene | 97 | 006874-10-8 |
| 13 | 11.237 | 0.24 | Gamma. -Terpinene | 96 | 000099-85-4 |
| 14 | 13.098 | 0.07 | Linalool | 93 | 000078-70-6 |
| 15 | 15.058 | 10.24 | Camphor | 98 | 000076-22-2 |
| 16 | 15.436 | 0.04 | L-Menthone | 97 | 010458-14-7 |
| 17 | 15.572 | 0.06 | Isoborneol | 94 | 000124-76-5 |
| 18 | 16.033 | 3.90 | Borneol L | 97 | 000464-45-9 |
| 19 | 16.337 | 0.05 | Menthol, | 91 | 015356-70-4 |
| 20 | 16.521 | 0.22 | 4-Terpineol | 96 | 000562-74-3 |
| 21 | 17.171 | 1.44 | Alpha. Terpineol | 91 | 000098-55-5 |
| 22 | 19.514 | 0.25 | Pulegone | 98 | 015932-80-6 |
| 23 | 21.925 | 1.08 | L-Bornyl Acetate | 99 | 005655-61-8 |
| 24 | 27.455 | 0.66 | Trans-Caryophyllene | 99 | 000087-44-5 |
| 25 | 28.698 | 0.08 | Alpha. -Humulene | 96 | 006753-98-6 |

**Quantitative analysis of the ovarian follicles:**

The results of ovarian follicles counting in control and after treatment with rosemary oil extract are shown in (Table 2). Our study showed that the ovary tissue in the control group consisted of different growing follicles in various stages, primary secondary, tertiary, atretic, graffian follicles and corpus luteum. The primary follicles surrounded by one layer of granulosa cuboidal cells, while secondary follicles surrounded by more than one layer of cuboidal granulosa cells. Tertiary follicles with their antrum which is a fluid filled cavity. Atretic follicles are characterized by numerous pyknotic nuclei in the granulosa. While corpus luteum is made up of lutein cells. Fig. (1: a, b).

Histological sections of the ovary treated with doses 250, 500, 1000 mg oil extract/kg body weight of rosemary showed a significant decrease $P<0.05$ in all follicles compared with control group Fig. (2, 3 and 4), especially the number of graffian follicles and corpora lutea, as well as presence increased significantly in the number of atretic follicles (Table 2).

**Table 2: The number of follicle types in the control and rosemary oil-treated groups.**

| Rosemary oil doses and control | Primary Follicles | Secondary Follicles | Atretic follicles | Graffian follicles | Corpus luteum |
|---|---|---|---|---|---|
| Control without rosemary oil | 115.38±3.27a | 46.43±2.2a | 10.25±0.46a | 7.90±0.23a | 14.98±0.5a |
| 250 | 108.67±2.58ab | 35.80±1.15b | 11.65±0.57b | 5.48±0.35b | 11.78±0.64b |
| 500 | 103.32±2.8b | 29.77±1.08c | 12.17±0.38b | 4.15±0.23c | 10.72±0.49b |
| 1000 | 91.23±2.85c | 19.02±0.65d | 14.50±0.24c | 2.53±0.27d | 5.65±0.37c |

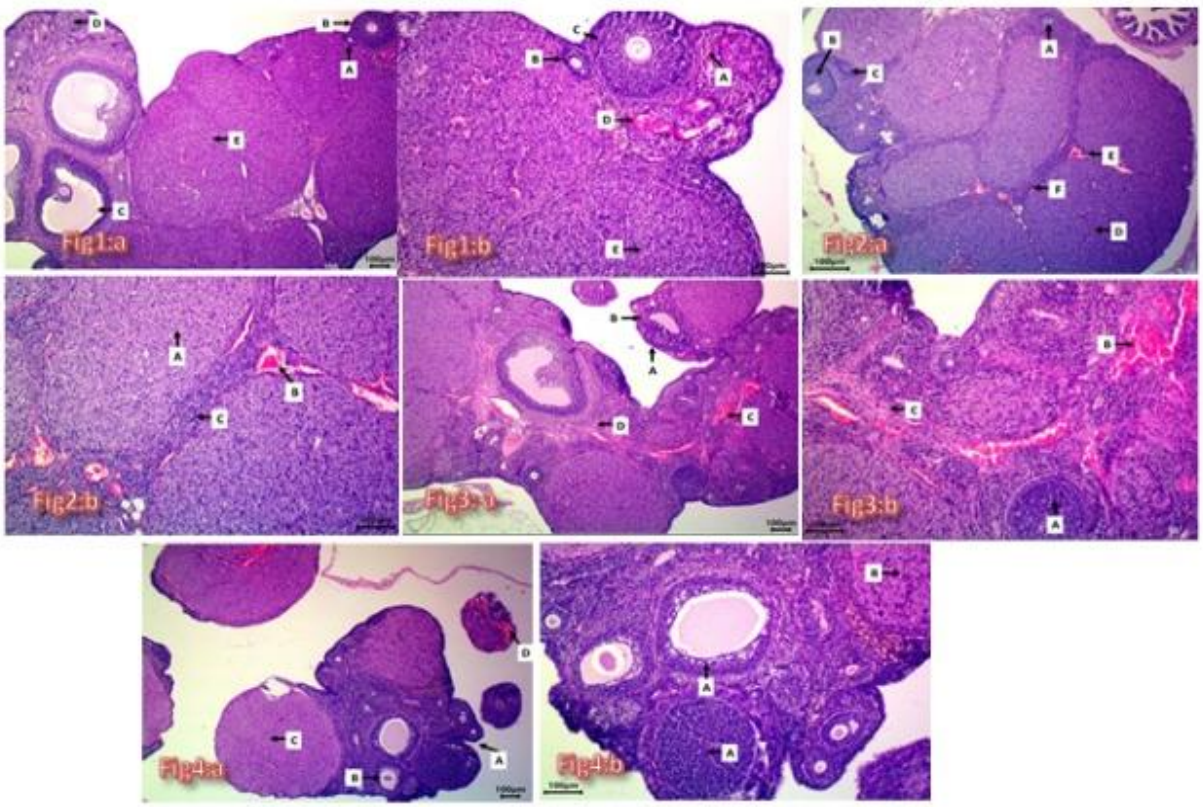

**Fig. 1a: Photomicrograph of rat ovary of control group showing the primary (A), secondary (B), tertiary (C), atretic follicles (D) and corpora luteum (E). 40X.**

**Fig. 1b: Photomicrograph of rat ovary of control group showing the primordial (A), primary (B), secondary (C), corpora luteum (E) and congested blood vessels (F). H and E stain, 100X.**

**Fig. 2a: Photomicrograph of rat ovary of the 250-rosemary treated group showing the abnormal primary (A) and secondary follicles (B), with increased atretic follicles (D) corpora luteum (E), congested blood vessels (E) and increased fibrous tissue (F). H and E stain, 40X.**

**Fig. 2b: Photomicrograph of rat ovary of the 250-rosemary treated group showing increased corpora luteum (A), congested blood vessels (B) and increased fibrous tissue (C). H and E stain, 100X.**

**Fig. 3a: Photomicrograph of rat ovary of the 500-rosemary treated group showing the ovarian atrophy (A), increased atretic follicles (B) severe congested blood vessels (C) and increased fibrous tissue (D). H and E stain, 40X.**

**Fig. 3b: Photomicrograph of rat ovary of the 500-rosemary treated group showing the increased atretic follicles (A) severe congested blood vessels (B) and increased fibrous tissue (C). H and E stain, 100X.**

**Fig. 4a: Photomicrograph of rat ovary of the 1000 rosemary treated group showing the severe ovarian atrophy (A), increased atretic follicles (B) and corpora luteum (C), severe congested blood vessels (D). H and E stain, 40X.**

**Fig. 4b: Photomicrograph of rat ovary of the 1000 rosemary treated group showing the increased atretic follicles (A) and corpora luteum (B). H and E stain, 100X.**

**Histopathological changes**

The current study's findings revealed histological changes of varying severity, which increased with increasing extract dose. Microscopic examination of normal ovarian tissue in the control group of suckling rats showed that it consisted of different stages of development: Primary, secondary and mature with corpus luteum Fig. (5). The second group of suckling rats, which was given a dose of 250 mg rosemary oil/kg body weight for 21 days, demonstrated several histological changes, including the deposition of an acidic substance in the interstitial tissue of the ovary, increased atretic follicles and blood vessel congestion Fig. (6: a), as well as infiltration of mononuclear inflammatory cells, vascular degeneration of luteal cells at the center of the corpus luteum, with intercellular oedema, and an increase in fibrous tissue Fig. (6: b), compared with the normal ovary control group Fig. (5).

These changes were more severe in the third and fourth groups, which received 500 and 1000 mg/kg body weight of suckling rats for 21 days, and included diffuse degeneration of the various ovarian follicles, oedema Fig. (7: a), coagulative necrosis of the corpus luteum center, and hemorrhage Fig. (7: b), compared to the control group's normal ovary structure Fig. (5). Diffuse ovarian follicle degeneration, proteinuric oedema with an acidic appearance, hemorrhagic cysts, fibroblast hyperplasia, and an increase in collagen fibres deposition in ovarian tissue with the occurrence of fat replacement Fig. (8: a, b), compared to the normal ovary structure in the control group Fig. (5).

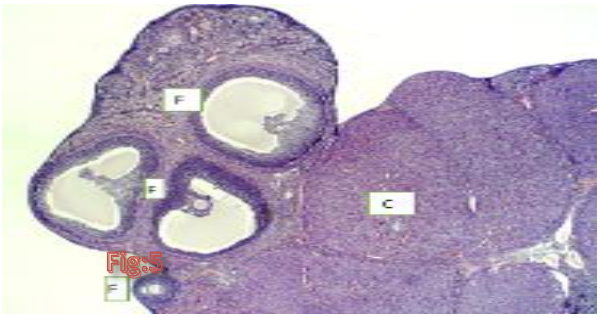


**Fig. 5: Control, normal histological structure of the ovary in the suckling rats. Shows the corpus luteum (c), and ovarian follicles in different stages (F). H and E stains, 100X.**

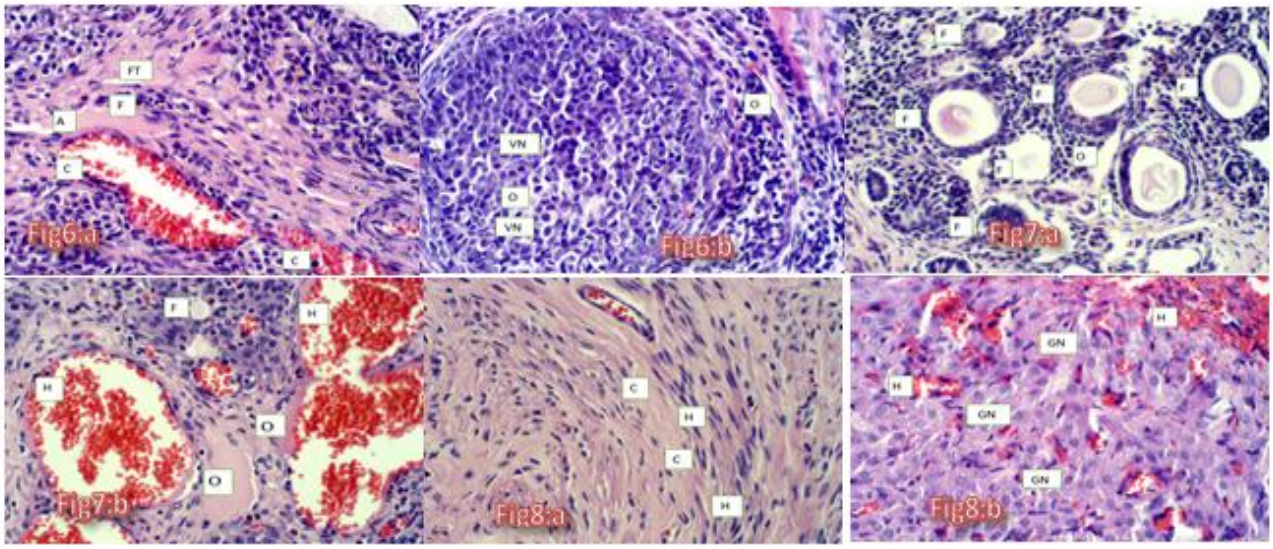


**Fig. 6: Histological structure of the ovary in the suckling rats treated with 250 mg rosemary/kg. b.w. HE stains, 400X. (a): Deposition of acidic substance in the ovarian interstitial tissue (A),**

**congestion in blood vessels (C), infiltration of mononuclear inflammatory cells (F), fibrous tissue (FT). (b): Vascular degeneration of luteal cells at the corpus luteum centre (VN), intercellular Oedema (O).**

**Fig. 7: Histological structure of the ovary in the suckling rats treated with 500 mg rosemary/kg. b.w. HE stains, 400X. (a): Diffuse degeneration of the various ovarian follicles (D), oedema (O). (b): Coagulative necrosis of the corpus luteum center (CN). Hemorrhage (H).**

**Fig. 8: Histological structure of the ovary in the suckling rats treated with 1000 mg rosemary/kg. b.w. HE stains, 400X. (a): Diffuse degeneration of the ovarian follicles (D), proteinuric edema that took an acidic appearance (A), hemorrhagic cysts (H). (b): Fibroblast hyperplasia (H), deposition of collagen fibres in ovarian tissue (C).**

Our findings in the mammary gland revealed that mammary gland tissue in the control group is organized in lobules composed of branched ducts and terminal secretory alveoli surrounded by interstitial connective tissue Fig. (9). In comparison to the control group, the other groups had histopathological lesions of the mammary gland of suckling rats. Mucous degeneration in the living cells of the lactating secretory alveoli, with milk substance accumulation in these alveoli ducts and edema between the glandular structures, was observed in the second group after 21 days of treatment with 250 mg rosemary oil/kg b.w Fig. (10). These changes increased with increasing the dose of the volatile oil extract, with the third and fourth groups receiving 500 and 1000 mg rosemary oil/kg b.w. for 21 days, atrophy in the degenerated lactating alveoli, deposition of collagen fibers between these alveoli, and the occurrence of the fatty replacement phenomenon Fig. (11, 12).

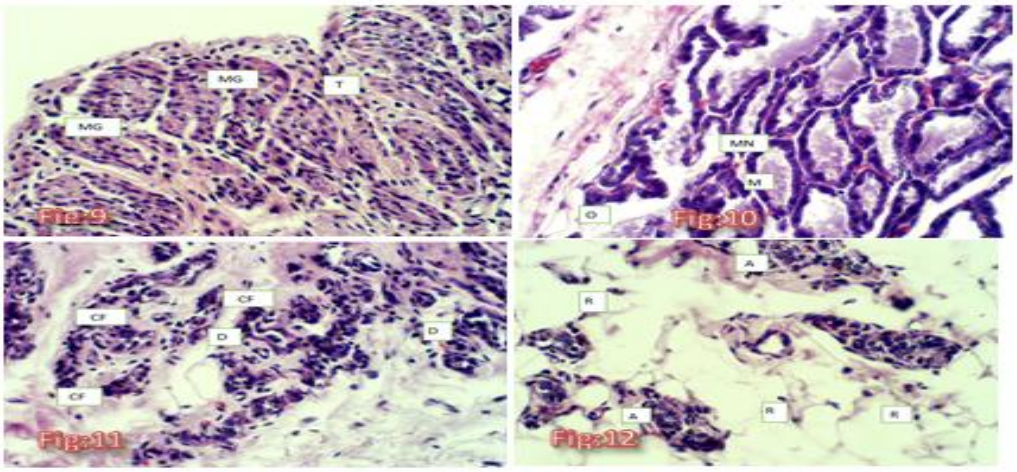


**Fig. 9: Control, normal histological structure of the mammary gland in the suckling rats (MG), and the surrounding interstitial (T). HE stains 400x.**

**Fig. 10: Histological structure of the mammary gland in the suckling rats treated with 250 mg rosemary/kg. b.w. HE stain 400x. Mucous degeneration in the lining cells of the lactating secretory alveoli (arrow), accumulation of milk substance in the alveoli ducts (M), oedema between the glandular structures (O).**

**Fig. 11: Histological structure of the mammary gland in the suckling rats treated with 500 mg rosemary/kg. b.w. HE stains 400x. Atrophy in the degenerated lactating alveoli (A), deposition of collagen fibres between these alveoli (C).**

**Fig. 12: Histological structure of the mammary gland in the suckling rats treated with 500 mg rosemary/kg. b.w. HE stains 400x. Atrophy in the degenerated lactating alveoli (A), fatty replacement phenomenon (R).**

## DISCUSSION

Some essential oils and their derivatives are known to cause abortion, reproductive hormone modulation, maternal toxicity, teratogenicity, milk production, and embryo-fetotoxicity (Dosoky and Setzer, 2021).

In our study, the results revealed that the rosemary oil extract which was obtained via hydro distillation and analyzed using GC/MS, shows the major components were 1,8-cineole 48.49%, alpha-pinene 12.57%, camphor 10.24%, beta-pinene 8.22, camphene 5.44, cymene 4.33, borneol L 3.90, α-terpineol 1.44%, beta-myrcene 1.28%, l-bornyl acetate 1.08%, as well as other components were reported such as α-fenchene, trans-caryophyllene, α-thujene, pulegone, 4-terpineol, l-phellandrene, α-thujene, gamma-terpinene, α-terpinene, and linalool (Table 1). Other findings recorded a similar chemical substances of rosemary oil (Mossa *et al.*, 2019) reported that the main major substances of rosemary oil is 31.45% 1,8-cineole, 11-8.86% borneol, 1.01 % α-pinene, 8.86% linalool, 7.32% camphor, 3.37% linalyl acetate, 3.32% α-terpineol, 3.42% Y-terpinene and 1.82% cymene. In addition, our results about the components of rosemary oil were agreement with (Kocak *et al.*, 2021). The essential oil content and composition are not constant, but change in response to growth conditions and environmental factors (Ahmad *et al*., 2018; Ashraf *et al*., 2018; Kulak *et al*., 2019).

Rosemary oil extract prepared in our study is mainly composed of 48.49% 1,8 ceneol or eucalyptol, is oxide monoterpene present in the mainly oil of several plants. Ceneol has many therapeutically actions such as anti-proliferative, anti-inflammatory, anti-microbial and antioxidant activity (Mahajan *et al*., 2020; Lešnik *et al*., 2021; Cai *et al*., 2021). It has been demonstrated that 1,8-cineole inhibits cell proliferation in a dose dependent manner. Its mechanism of action is apoptosis induction. As a result, the effect observed in the current study could be attributed to this component. Another study on reproductive toxicity discovered that the highest ceneol doses reduced the number of corpora lutea, insertion sites, the mass of pregnant rat fetuses (pre-implantation), and the number of dead fetuses in rats (Galdas *et al*., 2016). Cineol, on the other hand, affects males as well (Nusier *et al*., 2007) sperm motility at a dose of 500 mg/Kg b.w. In the cauda epididymis, sperm density, seminiferous tubule diameter, Leydig cell nuclear diameter, and epithelial cell height in the cauda and caput epididymides, and seminal vesicles were significantly lower in treatment. Furthermore, in doses of 50 and 100 mg/kg b.w., *Rosmarinus officinalis* extract administration resulted in a significant decrease in the germinal cell population, testosterone, spermatocytes (primary and secondary), spermatids, and spermatozoa (Heidari-Vala *et al*., 2013).

In morphometric analysis, the high doses of rosemary oil extract used in this study are most likely to blame for the histochanges in the ovary and mammary gland. Ovarian toxicity can be determined using follicle quantitation and morphometric analysis (Smith *et al*., 1991). The study of follicular numbers could provide important information about ovarian function, specifically the relationship between folliculogesis and the factors that regulate it (Myers *et al*., 2004). Our result revealed that the administration of rosemary essential oil with doses 250,500 and 1000mg/kg b.w. for 21 days (suckling period) decreased the number of (primary, secondary, graffian) follicles and corpora luteum with increased the number of atretic follicles, most of the ovarian follicles undergo atresia, a hormonally-controlled apoptotic process.

Our findings are consistent with those of (Motaghi *et al.*, 2020), who found that chronic long-term administration of Rosemary officinalis essential oil reduces the number of graffian follicles and corpora lutea. Estrogen is needed for follicle development and growth, and estrogen therapy promotes granulosa cell division and increases ovarian weight. Because of the increased metabolism of endogenous, endogenous estrogen, (Zhu *et al*., 1998). *Rosemary officinalis* inhibited the proliferation of ovarian cancer cell lines by affecting the cell cycle at multiple stages, according to (Tia *et al*., 2012). It induced apoptosis by modifying the expression of multiple apoptosis-regulating genes, including Bcl-XL, Bcl-2, clAP-1, HIF and HO-1. As well as rosemary oil extract is cytotoxic to breast cancer cells by inhibiting the expression of BCL-XL, an anti-apoptotic gene (Shakhseniaie *et al*., 2020).

The pathological findings revealed that taking 250mg rosemary oil/kg b.w. for 21 days caused mild ovarian and mammary gland damage, whereas taking 500 and 1000mg/kg rosemary oil caused severe ovarian and mammary gland damage, with main lesions of severe blood vessels congestion, collagen fibers deposition, high atretic follicles, high corpora lutea, ovarian atrophy, vascular degeneration, coagulative necrosis, edema, degenerated lactating alveoli and fatty replacement phenomenon. The occurrence of such pathological lesions may be due to the liberation of high effective free radical types as a result of administering high doses of rosemary oil extract, as previous research demonstrated that administering rosemary resulted in a significant increase in ROS, and high levels of ROS could result in necrosis cell death. Intoxication with high doses of rosemary oil caused pathological and functional damage in rat's ovary and mammary gland; these changes may be related to hormonal alteration. According to the previous work, camphor oil administration increased plasma FSH and LH, implying stimulation hypothalamic-pituitary. Camphor oil has been shown in previous studies to be a fertility reducer. Furthermore, so that camphor oil has an estrogenic effect by increasing progesterone and estrogen levels (Seidlova-Wuttke *et al*., 2006). Follicle stimulation hormone (FSH) stimulates estrogen synthesis and secretion, which is controlled by hypothalamic gonadotropin releasing hormone (GnRH). FSH stimulates the follicle to release estrogens in sexually mature females. Camphor oil treatment, on the other hand, reduced prolactin levels. Camphor is well known for its use in modulating sexual activity, contraception, inducing abortion, and reducing lactating women's milk production (Nelson *et al*., 2019). Several studies have discovered that camphor components can interact with gonadotropins and gonadal hormones, causing adolescent retardation and a decrease in reproductive organ volumes in both sexes (Schlumpf *et al*., 2004). Various ultraviolet filters containing up to 4% camphor (4-MBC) exhibited estrogenic effects when tested on animals (Schlumpf *et al*., 2008). Camphor can cross the placenta and cause abortion (Rabi *et al*., 1997).

High doses of 1,8 Ceneole, on the other hand, caused a change in the ultrastructure of mitochondria, endoplasmic reticulum, and other membrane structures in mice liver and kidneys. Camphor is the second main active substance of rosemary oil extract and has toxic effects on the female reproductive system. A previous study found that injecting camphor (5, 10 and 20mg/kg) into rats caused epithelial degeneration, fewer uterine glands, uterus lumen enlargement, and increases in estrogen concentration, all of which affect rat fertility and may result in animal abortion (Al-qudsi and Linjawi, 2012). Camphor at various concentrations (50,75mg/kg b.w.) causes cytotoxicity, non-active tissue oocyte detachment, inhibition of ovaries follicles, and hemorrhage in rats (Al-Fartosi *et al.,* 2017). Camphor can be detected in the mother's blood 15 minutes after swallowing through delivery 36 hours later. It was also found in amniotic fluid, the umbilical cord, and the blood of a fetus that was unable to breathe (Manoguerra *et al*., 2006). 9 to 19 children were becoming epileptic 14-120 minutes after swallowed 1000mg/wing 0.07 to 0.6 gm of camphor (Ford *et al.,* 2001).

Other research's team have reported that rosemary oil is toxic (Gokturk *et al.,* 2020) utilizing *R. officinalis* essential oil at a dosage of 20L for 24 hours or 10L after 48 hours will be more accurate because these dosages cause 100% death in the insect. Damasco and Lemonica (1999) found a high rate of abnormal embryos in rats given 260 mg/kg rosemary ethanolic extract from the first day to the fourth day of gestation. During the same period, higher dose (1040 mg/kg) rosemary reduced the number of blastocysts found in the uterus, because chromosome breaks and detections are important factors responsible for abortion in humans and animals, the genotoxic and mutagenic effects of rosemary oil could be responsible for the embryotoxic effect in mice reported in the preceding studies. Rosemary oil at doses (300, 1000 or 2000 mg/kg) induced significant increases in DNA damage in the mouse cells (Maistro and Goulart, 2010).

## CONCLUSIONS

The study suggested that the administration of high doses of *R. officinalis* oil can have an impact on the fertility of exposed animals, as a result, the use of rosemary oil in paediatrics and pregnant or suckling women should be approached with caution due to the toxicity.

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## REFERENCES

Abu-Al-Basal, M.A. (2010). Healing potential of *Rosmarinus officinalis* L. on full-thickness excision cutaneous wounds in alloxan-induced-diabetic BALB/c mice. *J. Ethnoph.,* **131**(2), 443-450. DOI: 10.1016/j.jep.2010.07.007

Ahmad, P.; Ahanger, M.A.; Singh, V.P.; Tripathi, D.K.; Alam, P.; Alyemeni, M.N. (2018). "Plant Metabolites and Regulation under Environmental Stress". Academic Press, Elsevier, 125p. London Wall, London EC2Y 5AS, UK. DOI: org/10.1016/B978-0-12-812689-9.00006-6

Al-Fartosi, K.G.; Alomer, D.K.; Al-Muswiec, R.T. (2017). Toxopathological effects of camphor on some organs of female rats. *Iraq Med. J.*, **1**(4), 106-109. DOI: 10.22371/imj.12201706

Al-Qudsi, F.; Linjawi, S. (2012). Histological and hormonal changes in rat endometrium under the effect of camphor. *L. Sci. J.*, **9**(2), 348-355.

Altinier, G.; Sosa, S.; Aquino, R.P.; Mencherini, T.; Loggia, R.D.; Tubaro, A. (2007). Characterization of topical antiinflammatory compounds in *Rosmarinus officinalis* L. *J. Agric. Food Chem.*, **55**(5), 1718-1723. DOI: 10.1021/jf062610

Andrade, J.M.; Faustino, C.; Garcia, C.; Ladeiras, D.; Reis, C.P.; Rijo, P. (2018). *Rosmarinus officinalis* L.: An update review of its phytochemistry and biological activity. *Fut. Sci. OA*, **4**(4), FSO283. DOI: 10.4155/fsoa-2017-0124

Ariffin, N.H.M.; Hasham, R. (2016). Potential dermatological application on Asian plants. *Biotech. Biopr. Eng.*, **21**, 337-354. DOI: 10.1007/s12257-015-0750-4

Ashraf, M.A.; Iqbal, M.; Rasheed, R.; Hussain, I.; Riaz, M.; Arif, M.S.; (2018). Environmental stress and secondary metabolites in plants: An overview. *P. Meta. Reg. Envir. Str.*, 153-167. DOI: 10.1016/B978-0-12-812689-9.00008-X

Begum, A.; Sandhya, S.; Vinod, K.R.; Reddy, S.; Banji, D. (2013). An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). *Acta Scient. Polon. Tech. Alimen.*, **12**(1), 61-73.

Bouyahya, A.; Belmehdi, O.; Benjouad, A.; El-Hassani, R.A.; Amzazi, S.; Dakka, N.; Bakri, Y.; (2020). Pharmacological properties and mechanism insights of Moroccan anticancer medicinal plants: What are the next steps? *Ind. Crops Prod*., **147**, 112198. DOI: 10.1016/j.indcrop.2020.112198

Cai, Z.; Peng, J.; Chen, Y.; Tao, L.; Zhang, Y.; Fu, Y.; Long, Q.; Shen, X. (2021). 1,8-Cineole: A review of source, biological activities, and application. *J. Asian Nat. Prod. Res*., **23**(10), 938-954. DOI: 10.1080/10286020.2020.1839432

Caldas, G.F.R.; Limeira, M.M.F.; Aramjo, A.V.; Wanderley, A.G.; Albuquerque, G.S.; Silva-Neto, J.C. (2016). Repeated-doses and reproductive toxicity studies of the monoterpene 1,8-cineole (eucalyptol) in Wistar rats. *Food Chem. Toxic*., **97**, 297-306 DOI: org/10.1016/j.fct.2016.09.020

Damasco, D.C.; Lemonica, I.P. (1999). Embryotoxicity and anti-implantation effects of rosemary (*Rosmarinus officinalis* L.) extract in pregnant rats within preimplantation period. *Rev. Bras. Toxi.*, **12**(02), 47-54.

De Freitas Junior, L.M.; de Almeida E.B. (2013). Medicinal plants for the treatment of obesity: Ethnopharmacological approach and chemical and biological studies. *American J. Trans. Res.*, **9**(5), 2050-2064.

De Oliveira, J.R.; Camargo, S.E.A.; de Oliveira, L.D. (2019). *Rosmarinus officinalis* L. (rosemary) as therapeutic and prophylactic agent. *J. Biomed. Sci.* **26**(1), 5. DOI: 10.1186/s12929-019-0499-8

Dosoky, N.S.; Setzer, W.N. (2021). Maternal reproductive toxicity of some essential oils and their constituents. *Int. J. Mol. Sci.* **22**(5), 2380. DOI: org/10.3390/ ijms22052380

Ford, M.D.D.; Ling, L.J.; Erickson, T. (2001). Clinical toxicology., Philadelphia, W.B. Saunders Company, *J. Emer. Med*., 345. DOI: 10.1016/S0736-4679(02)00469-9

Gokturk, T.; Kordali, S.; Ak, K.; Kesdek, M.; Bozhuyuk, A.U. (2020). Insecticidal effects of some essential oils against *Tribolium confusum* (du Val.) and *Acanthoscelides obtectus* (Say), (Coleoptera: Tenebrionidae and Bruchidae) adults. *Inter. J. Trop. Insect Sci*., **40**, 637-643. DOI: org/10.1007/s42690-020-00113-y

González-Minero, F.J.; Bravo-Díaz, L.; Ayala-Gómez, A. (2020). *Rosmarinus officinalis* L. (Rosemary): An ancient plant with uses in personal healthcare and cosmetics. *Cosm*., **7**(4),77. DOI.org/10.3390/cosmetics7040077

Heidari-Vala, H.; Hariry, R.E.; Sadeghi, M.R.; Akhondi, M.M.; Novin, M.G.; Heidaria, M. (2013). Evaluation of an aqueous-ethanolic extract from *Rosmarinus officinalis* (Rosemary) for its activity on the hormonal and cellular function of testes in adult male rat. *Iran J. Pharm. Res.*, **12**(2), 445-451.

Ielciu, I.; Niculae, M.; Pall, E.; Barbalat, C.; Tomut, I.; Olah, N.K.; Burtescu, R.F.; Benedec, D.; Oniga, I.; Hanganu, D. (2022). Antiproliferative and antimicrobial effects of *Rosmarinus officinalis* L. loaded liposomes. *Mole*., **27**(13), 3988. DOI: org/10.3390/

Jalali-Heravi, M.; Moazeni, R.S.; Sereshti, H. (2011). Analysis of Iranian rosemary essential oil: Application of gas chromatography-mass spectrometry combined with chemometrics. *J. Chromat.*, **1218**(18), 2569-2576. DOI: org/10.1016/j.chroma.2011.02.048

Kocak, M.Z.; Karadag, M.; Celikcan, F. (2021). Essential oil composition of salvia officinalis and rosmarinus officinalis. *J. Agri.*, **4**(1), 39-47. DOI: 10.46876/ja.938170

Kulak, M.; (2019). A time-course study on essential oil of rosemary (*Rosmarinus officinalis*) under drought stress. *Adıyaman Üniver. Bilim. Derg*, **9**(1), 165-189. DOI: 0000-0003-3673-9221

Lee, D.Y.; Hwang, C.J.; Choi, J.Y.; Park, M.H.; Song, M.J.; Oh, K.W.; Son, D.J.; Lee, S.H.; Han, S.B.; Hong, J.T. (2017). Inhibitory effect of carnosol on phthalic anhydride-induced atopic dermatitis via inhibition of STAT3. *Biomole. Therap.*, **25**(5), 535-544. DOI: 10.4062/bio mother. 006

Lemonica, I.P.; Damasceno, D.C.; di-Stasi, L.C. (1996). Study of the embryotoxic effects of an extract of rosemary (*Rosmarinus officinalis* L.). *Braz. J. Med. Bio. Res.* **29**(2), 223-227.

Lešnik, S.; Furlan, V.; Bren, U. (2021). Rosemary (*Rosmarinus officinalis* L.) extraction techniques, analytical methods and health-promoting biological effects. *Phytoch. Rev.*, **20**(6), 1273-1328. DOI: 10.1007/s11101-021-09745-5

Mahajan, M.; Kuiry, R.; Pal, P.K.; (2020). Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants. *J. App. Res. Med. Arom. Plants.*, **18**, 100255. DOI: org/10.1016/j.jarmap.2020.100255

Maistro, E.L.; Mota, S.F.; Lima, E.B.; Bernardes, B.M.; Goulart, F.C. (2010). Genotoxicity and mutagenicity of *Rosmarinus officinalis* (Labiatae) essential oil in mammalian cells *in vivo*. *Genet. Mol. Res.*, **9**(4), 2113-2122. DOI: 10.4238/vol9-4gmr857

Manoguerra, A.S.; Erdman, A.R.; Wax, P.M.; Nelson, L.S.; Caravati, M.E.; Cobaugh, D.J.; Chyka, P.A.; Olson, K.R.; Booze, L.L.; Woolf, A.D.; Keyes, D.C.; Christianson, G.; Scharman, E.J.; Troutman, W.G. (2006). Camphor poisoning: An evidence-based practice guideline for out-of-hospital management. *Clinic. Toxicol.*, **44**(4), 357-370. DOI: 10.1080/15563650600671696

Mohammed, I.A.; Shaban, K.A.; Albadrany, Y.M. (2022). Hepato-renal and hematological effects of flunixin and silymarin coadministration in rats. *Iraq. J. Veter. Sci*., **36**(2), 367-373. DOI: 10.33899/IJVS.2021.130323.1800

Mossa, A.H.; Afia, S.I.; Mohafrash, S.M.M.; Abou-Awad, B.A. (2019). Rosemary essential oil nanoemulsion, formulation, characterization and acaricidal activity against the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *J. P. Prot. Res.*, **59**(1), 102-112. DOI: 10.24425/jppr.2019.126039

Motaghi, S.; Ahmadusefi, N.; Shohreh, B.; Nazem, M. (2020). Chronic administration of rosmarinus officinalis essential oil decreases fertility in female rats. *J. Morph. Sci.*, **37**, 88-91. DOI: org/ 10.51929/jms.37.15.2020

Myers, M.; Britt, K.L.; Wreford, N.G.; Ebling, F.J.; Kerr, J.B. (2004). Methods for quantifying follicular numbers within the mouse ovary. *Reprod.*, **127**(05), 569-580. DOI: 10.1530/rep.1.00095

Nelson, L.S.; Howland, M.; Lewin, N.A.; Smith, S.W.; Goldfrank, L.R.; Hoffman, R.S. (2019). "Goldfrank's Toxicologic Emergencies". 11th ed., McGraw-Hill Education.

Nguyen, H.C.; Nguyen, H.N.T.; Huang, M.Y.; Lin, K.H.; Pham, D.C.; Tran, Y.B.; Su, C.H. (2021). Optimization of aqueous enzyme-assisted extraction of rosmarinic acid from rosemary (*Rosmarinus officinalis* L.) leaves and the antioxidant activity of the extract. *J. Food Proc. Preserv.*, **45**(3), e15221. DOI: 10.1111/jfpp.15221

Nuiser, M.K.; Bataineh, H.N.; Daradkah, H.M. (2007). Adverse effect of rosemary (*Rosmarinus officinalis* L.) on reproductive function in adult male rats. *Exp. Bio. Med.*, **232**(6), 809-813. DOI:10.3181/00379727-232-2320809

Rabi, W.; Katzgraber, F.; Steinlechner, M. (1997). Camphor ingestion for abortion (case report), *Foren. Sci. Inter*., **89**(1-2) 137-140.

Ribeiro-Santos, R.; Carvalho-Costa, D.; Cavaleiro, C.; Costa, H.S.; Albuquerque, T.G.; Castilho, M.C.; Ramos, F.; Melo, N.R.; Sanches-Silva, A. (2015). A novel insight on an ancient aromatic plant: The rosemary (*Rosmarinus officinalis* L.). *Trends Food Sci. Tech.* **45**(2), 355-368. DOI: 10.1016/j.tifs.2015.07.015

Sales, A.J.; Pashazadeh, M. (2020). Study of chemical composition and antimicrobial properties of rosemary (*Rosmarinus officinalis*) essential oil on *Staphylococcus aureus* and *Escherichia coli in vitro*. *Inter. J. Life Sci. Biotech*., **3**(1), 62-69. DOI: 10.38001/ijlsb.693371

Schlumpf, M.; Durrer, S.; Faass, O.; Ehnes, C.; Fuetsch, M.; Gaille, C.; Henseler, M.; Hofkamp, L.; Maerkel, K.; Reolon, S.; Timms, B.; Tresguerres, J.A.F.; Lichtensteiger, W. (2008). Developmental toxicity of UV filters and environmental exposure: A review. *Inter. J. Andr.*, **31**(2), 144-151. DOI: 10.1111/j.1365-2605.2007.00856.x

Schlumpf, M.; Jarry, H.; Wuttke, W.; Ma, R.; Lichtensteiger, W. (2004). Estrogenic activity and estrogen receptor beta binding of the UV filter 3-benzylidene camphor comparison with 4-methyl benzylidene camphor. Comparison with 4-methyl benzylidene camphor. *Toxic.*, **199**(2-3), 109-120. DOI: 10.1016/j.tox.2004.02.015

Seidlova´-Wuttke, D.; Jarry, H.; Christoffel, J.; Rimoldi, G.; Wuttke, W. (2006). Comparison of effects of estradiol (E2) with those of octylmethoxycinnamate (OMC) and 4-methylbenzylidene camphor (4-MBC)-2 filters of UV light-on several uterine, vaginal and bone parameters. *Toxic. App. Pharm*., **210**(3), 246-254. DOI: 10.1016/j.taap.2005.05.006

Seo, E.J.; Fischer, N.; Efferth, T. (2018). Phytochemicals as inhibitors of NF-kappaB for treatment of Alzheimer's disease. *Pharm. Res.*, **129**, 262-273. DOI: 10.1016/j.phrs.2017.11.030

Shakhseniaie, M.; Lotfabadi, N.N.; Haghirossadat, F. (2020). Effect of rosemary essential oil on the expression of BCL-XL, an anti-apoptotic gene, in MCF-7 cell line breast cancer. *Danesh. Med.*, **27**(4), 45-52.

Smith, B.J.; Plowchalk, D.R.; GlennSipes, I.G.; Mattison, D.R. (1991) . Comparison of random and serial sections in assessment of ovarian toxicity. *Reprod. Toxic.*, **5**(4), 379-383. DOI: org/10.1016/0890-6238(91)90097-Y

Soulaimani, B.; El Hidar, N.; El Fakir, S.B.; Mezrioui, N.; Hassani, L.; Abbad, A. (2021). Combined antibacterial activity of essential oils extracted from *Lavandula maroccana* (Murb.), *Thymus pallidus* Batt. and *Rosmarinus officinalis* L. against antibiotic-resistant Gram-negative bacteria. *European J. Integ. Med*., **43**, 10131.DOI: 10.1016/j.eujim.2021.101312

Survarna, S.K.; Christopher, L.; Bancroft, J.D. (2013). "Theory and Practice of Histological Technique". 3rd ed., NY. Churchill Livingstone. New York., pp.109-121.

Tai, J.; Cheung, S.; Wu, M.; Hasman, D. (2012). Antiproliferation effect of Rosemary (*Rosmarinus officinalis*) on human ovarian cancer cells *in vitro*. *Phytomed.*, **19**(5), 436-443. DOI: 10.1016/j.phymed.2011.12.012

Tian, X.F.; Yao, J.H.; Zhang, X.S. (2010). Protective effect of carnosol on lung injury induced by intestinal ischemia/reperfusion. *Sur. Today*, **40**(9), 858-865. DOI: 10.1007/s00595-009-4170-y

Zhu, B.T.; Loder, D.P.; Cai, M.X.; Ho, C.T.; Huang, M.T.; Conney, A.H. (1998). Dietary administration of an extract from rosemary leaves enhances the liver microsomal metabolism of endogenous estrogens and decreases their uterotropic action in CD-1mice. *Carcin.*, **19**(10), 1821–1827. DOI:org/10.1093/carcin/19.10.1821

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**التغيرات النسجية والشكلية في أنسجة المبيض والغدد اللبنية في أناث الجرذان المرضعة تحت تأثير المستخلص الزيتي لأكليل الجبل *Rosemarinus officinals***

**أسماء جبر بشير** **فاطمة قاسم محمد**

قسم علوم الحياة/ كلية العلوم/ جامعة الموصل/ موصل/ العراق

**القاسم ناظم أحمد حسن**

قسم الفسلجة والكيمياء الحياتية والأدوية/ كلية الطب البيطري/ جامعة الموصل/ موصل/ العراق

**الملخص**

أكليل الجبل *Rosemarinus officinals* نبات طبي استخدمه الناس في جميع أنحاء العالم منذ فترة طويلة، ولكن العديد من مكوناته قد تكون ضارة بالجسم. تبحث هذه الدراسة في تأثير مستخلص زيت اكليل الجبل على تركيب المبيض والغدة اللبنية لإناث جرذان Sprague Dawley. تم إجراء التحليل الكيميائي للزيت العطري المختبَر باستخدام كروماتوغرافيا الغاز جنبا الى جنب مع التحليل الطيفي الشامل Gas chromatography-mass spectrometry (GC-MS). أعطيت إناث الجرذان المرضعة عن طريق الفم مستخلص زيت *R. officinalis* بجرعات 250 و 500 و 1000 ملغم/كغم من وزن الجسم لمدة 21 يوماً، بينما أعطيت جرذان السيطرة زيت الذرة. بعد ذلك، تم الحصول على عينات من أنسجة هذه الأعضاء لدراسة التغيرات النسجية والقياسية الشكلية تم بعد ذلك تحديد عدد الجريبات الأولية والثانوية وكراف والجريبات المنحلة، والجسم الأصفر. عند المقارنة بمجموعة السيطرة فإن جميع جرعات زيت إكليل الجبل قللت بشكل كبير من عدد جريبات كراف والجسم الأصفر في المبيض، علاوة على ذلك، زاد عدد الجريبات المنحلة بشكل ملحوظ. وتمثلت التغيرات المرضية النسجية في أنسجة المبيض والغدة الثديية باحتقان شديد، ترسب ألياف الكولاجين، ضمور في المبيض، تنكس فجوي، نخر تخثري، وذمة، تنكس في الحويصلات الهوائية، وظاهرة الاستبدال الدهني. توضح الدراسة الحالية أن تأثيرات زيت إكليل الجبل على المبيض والغدد الثديية للإناث الجرذان يمكن أن تسبب تغيرات هيكلية كبيرة، وتزداد هذه التغييرات مع زيادة جرع زيت إكليل الجبل. يمكن أن يكون لذلك تأثير على الوظيفة الإنجابية وخصوبة الحيوانات، ونتيجة لذلك، يجب توخي الحذر عند استخدام زيت إكليل الجبل في طب الأطفال والنساء الحوامل أو المرضعات بسبب سمية زيت أكليل الجبل.

الكلمات الدالة: زيت أكليل الجبل، المبيض، الغدد اللبنية، التغيرات النسجية المرضية، التغيرات الشكلية القياسية.