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Original article

Consumption of sage (*Salvia officinalis*) promotes ovarian function by stimulating estradiol hormone release and controlling folliculogenesis, steroidogenesis, and autophagy

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ABSTRACT

Objectives: *Salvia officinalis* (sage) is widely sold in Saudi Arabia to make a popular herbal tea that women with reproductive disorders can drink daily. Despite the wide use of sage in traditional medicine, to our knowledge, little attention has been given to its effect on female reproduction. Our purpose was to understand the effects of sage extract on ovarian function to potentially shed light on its use in treating female infertility.

Methods: Forty female Wistar virgin female rats were randomly assigned to four groups, with 10 animals in each group. The control group received distilled water, and the three treatment groups received different concentrations of sage extract: 3.5, 15 or 60 mg/kg during 14 days. Ovarian tissues were cut into 5-μm sections and stained with hematoxylin and eosin. Plasma levels of reproductive steroids were measured using ELISA, and gene expression was examined via RT-PCR.

Results: Our results showed that low doses of sage (3.5 mg/kg and 15 mg/kg) have no effects on serum concentration levels of steroid hormone, the number of growing follicles, and on the transcripts of the steroidogenesis and folliculogenesis genes. In contrast, a high dose of the extract (60 mg/kg) significantly increased estradiol concentration levels. As a result, the number of growing follicles increased, while the number of abnormal follicles significantly decreased. Interestingly, sage extract also affected mRNA levels of genes involved in folliculogenesis and steroidogenesis processes. Two autophagy related genes, *LC3* and *ATG12*, were not affected, but all of the investigated genes, *INSL3, CYP17a, LHR, CCND2, IGF1, ESR1, ESR2, ACTB, GDF,* and *ATG5,* significantly increased with the highest dose of sage.

Conclusion: As a remedy, sage extract is best used at a high dose when treating female infertility disorders, as sage extract may promote ovarian function by stimulating folliculogenesis and steroidogenesis.

1. Introduction

Infertility is a major health and social problem that affects many people around the world (Lee et al., 2020b). There are differing opinions regarding the definition of infertility, but the World Health Organization (WHO) defines infertility as the inability to become or remain pregnant after one year of regular and unprotected sexual intercourse (WHO, 2017). Infertility affects approximately 48 million women globally and is caused by many factors. Twenty percent of infertility cases do not have an explanation. However, there are many known causes, including ovulation disorders, abnormal uterine function, obstruction of the fallopian tubes (Mascarenhas et al., 2012; Lee et al., 2020a), nutrition

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(Harrath et al., 2017), other diseases, and environmental effects (Lee et al., 2020b; Pivonello et al., 2020; Sirotkin et al., 2020). In Saudi Arabia and African cultures, infertility has a strong social meaning since marriage is considered to be successful only if a couple bears children. Thus, in most cases, women resort to complementary treatment with traditional therapies.

Salvia officinalis L. (Lamiaceae) has been used since ancient Egyptian civilization for increasing fertility in women, remedying sexual debility and treating menopausal and menstrual problems (Dweck, 2000). *S. officinalis* has been described in many ethnopharmacological reports (Martinez-Frances et al., 2017) and has been recommended for many gynecological diseases (Li et al., 2013). Therefore, this herb, known as "Maramia" in Saudi Arabia, is sold widely throughout the country and consumed as a tea known as Maramia tea. It is considered the "queen of herbs" since it is widely used in both medicinal and culinary preparations (Khan and Abourashed, 2010). The plant is well studied, and information on its biological activities has been widely reported (Martins et al., 2015; Ghorbani and Esmaeilizadeh, 2017; Ferreira Mendes et al., 2020). It has many antioxidant, anti-inflammatory, and antimicrobial activities against bacterial species, including *Staphylococcus aureus* and *Candida albicans* (Alizadeh, 2015). Sage essential oil has been traditionally used to treat many diseases in the nervous, circulatory, and respiratory systems (Radulescu et al., 2004; Loizzo et al., 2007), including bronchitis, persistent coughing, asthma, and angina (Khan et al., 2011; Walch et al., 2011).

Despite the widespread use of *Salvia officinalis* L. (Lamiaceae) in traditional medicine, to our knowledge, very little is known about the effect of this plant on female reproduction. Therefore, we studied the use of this plant as a traditional therapy for treating female reproductive disorders in Saudi Arabia and other countries to elucidate the effects of sage extracts on female ovarian functions. Specifically, we studied folliculogenesis and steroidogenesis as well as the potency of the plant extract in promoting female fertility. The findings of this study will be of significant interest as they will improve awareness regarding the effect of this plant on female fertility.

2. Materials and methods

2.1. Plant material

S. officinalis leaves were purchased in November 2018 from traditional medicine shops in Arar city, Saudi Arabia, and were then identified as being authentic by botanist colleagues at the Department of Faculty of Science in Arar, Saudi Arabia, under the voucher specimen code SO450420 and processed at the herbarium of the same department. At the laboratory, the leaves were weighed and then ground up.

2.2. Extraction

The *S. officinalis* leaves were ground, and the resulting powder (500 g) was successively extracted with hexane (to eliminate fixed oil) and then with dichloromethane (CH_2Cl_2) using a Soxhlet apparatus. The extracts were filtered with a Whatman filter. Then, the resulting solutions were evaporated using a rotary evaporator, which led to 12.36 g of n-hexane extract and 8.45 g of dichloromethane extract.

2.3. CH2Cl2 extract composition analysis

Gas chromatography (GC) and GC coupled with mass spectrometry (GC–MS/MS) (Thermo Trace GC Ultra/TSQ Quantum GC) were used to carry out CH_2Cl_2 analysis. The GC system was equipped with a split/splitless injector (220 °C), HP-5MS capillary column (TR5-MS, 60 m., 0.25 mm. id, and 0.25-μm film thickness), and flame-ionization detector (FID; 280 °C) and was coupled with an MS/MS detector, operating in the electron impact (EI) mode at 70 eV. We programmed the oven temperature to gradually increase from 50 °C to 280 °C in 10 °C/min increments and finally remain at 280 °C for 5 min (carrier gas = helium at 1.0 mL/min). The identification of the corresponding compounds was performed by using Wiley libraries and published literature (Adams, 2017) and by comparing their retention indices (RI) referenced from a series of n-alkanes and mass spectra from NIST/NBS.

2.4. Animal treatment

This study has been approved by the Scientific Research Ethics Committee at King Saud University (Reference No: KSU-SE-20-76). We used forty female Wistar rats that weighed 200–250 g and were obtained from the Animal Care Centre. They were housed as one per cage (22 °C to 24 °C) with a 12-h light/dark cycle and were allowed food and water *ad libitum*. The females were randomly assigned to four groups with 10 rats in each group. Group 1 was the control group, and the rats in this group were fed 1 mL of distilled water. The rats in group 2 were orally fed 3.5 mg/kg b.w./day of sage extract. The rats in group 3 were orally fed 15 mg/kg b.w./day of sage extract. The rats in group 4 were orally fed 60 mg/kg b.w./day of sage extract.

2.5. Histology

After 14 days of treatment, the animals were weighed and sacrificed. Their ovaries were extracted, weighed, and fixed in neutral buffered formalin (NBF) for 24 h. Tissues were cut into 5-μm-thick sections and stained with hematoxylin and eosin. The total follicle number for each ovary was calculated using the method described in our previous study (Harrath et al., 2017).

2.6. Hormone concentration assay

Blood samples were collected into lithium heparin tubes that were centrifuged at 3000 rpm for 10 min to obtain plasma. Progesterone, estradiol, and testosterone levels were detected using competitive enzyme-linked immunosorbent assays (ELISAs) following the manufacturer's instructions (Vector Laboratories, NJ, USA).

2.7. Analysis of gene expression

RNA was extracted from ovarian tissues that were previously stored in RNAlater stabilization reagent (Qiagen, Westburg, The Netherlands). For the RNA extraction, we used an RNeasy Mini Kit (Qiagen, Westburg, The Netherlands) with on-column DNase treatment using an RNase-Free DNase Set (Qiagen). Real-time PCR (RT-PCR) was carried out with SYBR green and an Applied Biosynthesis 7500 Fast RT-PCR system (Carlsbad, CA) with the gene-specific primers shown in Table 1. cDNA from these samples was acquired by RT-PCR and multiple primer sets using an iScript™ cDNA synthesis kit (Applied Biosystem, Carlsbad, CA) (Table 1).

2.8. Statistical analysis

Data are expressed as the means ± standard error of the mean (SEM). We used GraphPad Prism version 5 to determine the statistical significance of the differences in the mean values between the treatment groups and control group. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison was used for

Table 1
Primers for real-time RT-PCR.

Gene Symbol		Sequences
INSL3	Forward:	CTTCCTCACCAGGCTTCTCA
	Reverse:	CACCACCTGAGCCCTACAAT
CYP17a1	Forward:	ACTGAGGGTATCGTGGATGC
	Reverse:	TCGAACTTCTCCCTGCACTT
LHR	Forward:	TTATTCCGCCATCTTTGAGG
	Reverse:	ACAGGGGTTGAAAGCATCTG
CCND2	Forward:	CCTCACGACTTCATTGAGCA
	Reverse:	GGTAGCACACAGAGCGATGA
IGF-I	Forward:	CCGCTGAAGCCTACAAAGTC
	Reverse:	GGGAGGCTCCTCCTACATTC
ESR1	Forward:	CATCGATAAGAACCGGAGGA
	Reverse:	AAGGTTGGCAGCTCTCATGT
ESR2	Forward:	GAAGCTGAACCACCCAATGT
	Reverse:	CAGTCCCACCATTAGCACCT
ACTB	Forward:	AGCCATGTACGTAGCCATCC
	Reverse:	ACCCTCATAGATGGGCACAG
GDF9	Forward:	GATGTGACCTCCCTCCTTCA
	Reverse:	GCCTGGGTACTCGTGTCATT
ATG5	Forward:	CCTGAAGACGGAGAGAAGAAGAG
	Reverse:	CGGGAAGCAAGGGTGTCAT
LC3	Forward:	TGTTAGGCTTGCTCTTTTGG
	Reverse:	GCAGAGGAAATGACCACAGAT
ATG12	Forward:	CATTCTTACCTGGCGTTGAG
	Reverse:	CACTTCAAACCCTGTAATCC

statistical comparisons. When the p-value was ≤ 0.05, the difference was considered statistically significant.

3. Results

3.1. Analysis of CH2Cl2 sage extract

In the chemical analysis of the CH_2Cl_2 extract from *S. officinalis* by GC–MS/MS, 43 different chemical constituents grouped into different classes of chemical groups were identified (Table 2). These groups included the following: oxygenated monoterpenes (58.1%), monoterpene hydrocarbons (10.36%), sesquiterpene hydrocarbons (8.2%), oxygenated sesquiterpenes (6.79%), oxygenated diterpenes (13-epimanool, 6.45%), and phenolic diterpenes (carnosol, 1.27%). This analysis revealed that the dominant constituents (>1%) were 1,8-cineole (47.40%), 13-epimanool, (6.45%), 4,4 dimethylandrost-5-ene (5.90%), caryophyllene (5.11%), camphene (4.88%), α-pinene (4.40%), camphor (4.32%), β-pinene (2.94%), and ledol (2.32%) (Table 2).

3.2. Effect of sage extract on maternal body weight

We observed no differences in body weight between rats in the treatment groups and those in the control group (Fig. 1).

Table 2
Chemical analysis of the CH_2Cl_2 extract from *S. officinalis* by GC–MS/MS.

N°	Compound	MW[a]	RI[b]	Peak area (%)[c]	Identification
1	α-Pinene	136	938.2	4.40	RI, MS
2	Camphene	136	973.8	2.12	RI, MS
3	β-Pinene	136	965.6	2.94	RI, MS
4	E 2,3-Epoxycarane	152	1181	1.20	RI, MS
5	1,8-Cineole	154	1032	47.40	RI, MS
6	α-Thujone	152	1102	0.50	RI, MS
7	β-Thujone	152	1114	0.38	RI, MS
8	*trans*-Pinocarveol	152	1138	0.13	RI, MS
9	Camphor	152	1143	4.32	RI, MS
10	α-Terpineol	154	1192	0.82	RI, MS
11	Borneol	154	1188	0.35	RI, MS
12	Terpinen-4-ol	154	1187	0.32	RI, MS
13	β Fenchyl alcohol	154	1164	2.20	RI, MS
14	2-Hydroxycineole	170	1228	0.19	RI, MS
15	Linalyl acetate	196	1242	0.14	RI, MS
16	Thymol	150	1264	0.06	RI, MS
17	Bornyl acetate	196	1275	0.10	RI, MS
18	Carvacrol	150	1310	0.09	RI, MS
19	α-Terpinenyl acetate	196	1338	0.57	RI, MS
20	Caryophyllene	204	1436	5.11	RI, MS
21	Alloaromadendrene	204	1462	0.23	RI, MS
22	Calarene	204	1438	0.12	RI, MS
23	Aromadendrene	204	1435	1.53	RI, MS
24	α-Humulene	204	1442	0.78	RI, MS
25	Ledene	204	1484	0.43	RI, MS
26	Spathulenol	220	1557	0.36	RI, MS
27	Caryophyllene oxide	220	1575	0.55	RI, MS
28	Globulol	222	1582	0.08	RI, MS
29	Ledol	222	1586	2.32	RI, MS
30	Humulene-1,2-epoxide	220	1602	0.23	RI, MS
31	Tetracyclo[6.3.2.0(2,5)0.0(1,8)]tridecan-9-ol, 4,4-dimethyl-	220	2056	0.59	RI, MS
32	Alloaromadendrene oxide	220	1646	1.49	RI, MS
33	Arachidonic acid	304	2324	0.99	RI, MS
34	β-copaene	204	1430	0.12	RI, MS
35	Oleic Acid	282	2142	0.47	RI, MS
36	4,4,8Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	238	2342	0.48	RI, MS
37	Biformene	272	1985	0.13	RI, MS
38	n-Hexadecanoic acid	256	1963	0.65	RI, MS
39	13-Epimanool	290	2036	6.45	RI, MS
40	5β,7βH,10α-Eudesm-11-en-1α-ol	222	1625	0.69	RI, MS
41	9,12-Octadecadienoic acid	280	2130	0.55	RI, MS
42	4,4 dimethylandrost-5-ene	286	–	5.90	MS
43	Carnosol	330	–	1.27	MS

[a] MW: Molecular weight.
[b] RI: Retention index measured relative to n-alkanes (C8-C22) using HP-5MS column.
[c] Peak area (%): peak area of components in CH_2Cl_2 extract.

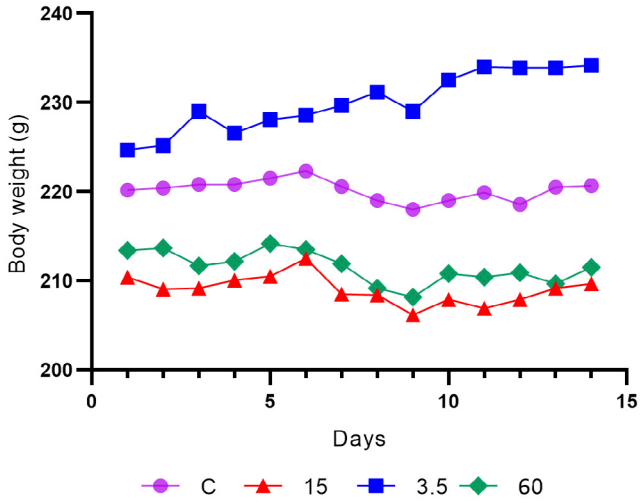

Fig. 1. Body weight changes over 14 days in the different groups. The 15 and 60 mg/kg doses decreased body weight compared to the control, but the changes were not statistically significant. Values are given as mean ± SEM. *Significant ($p < 0.05$) when compared to the control.

3.3. Effect of sage extract on steroid hormone secretion

The serum progesterone, estradiol, and testosterone levels of the four groups are shown in Fig. 2. No significant differences in progesterone or testosterone levels were observed between the treatment groups. Estradiol levels in the control group were significantly different from those in the group receiving 60 mg/kg sage. However, estradiol levels in the control group were not significantly different from those in the groups low-doses (3.5 and 15 mg/kg) sage extract groups.

3.4. Effects of sage extract on the number of follicles

The effects of aqueous sage extract on the number of ovarian follicles are presented in Fig. 3. While there were no significant differences in the number of primordial follicles in the treatment groups compared with that in the control group, there was a progressive increase in the number of growing follicles in the treatment groups compared to that in the control group. This increase was statistically significant only with the 60 mg/kg dose.

3.5. Effects of sage extract on the number of abnormal follicles

In this study, abnormal follicles in the ovary were defined as those containing multinucleate oocytes and/or multioocyte follicles (Fig. 4). We observed that sage extract significantly decreased the number of abnormal follicles in all treatment groups (3.5, 15 or 60 mg/kg) compared to the control group (Fig. 5).

3.6. Effect of sage extract on gene expression levels

We analyzed the effect of sage extract on ten genes involved in folliculogenesis, steroidogenesis, and autophagy (Fig. 6). Rats treated with a 60 mg/kg dose of sage extract had significantly increased mRNA levels of *INSL3, CYP17a, LHR, CCND2, IGF1, ESR1, ESR2, ACTB, GDF,* and *ATG5* genes (Fig. 6 A, B, C, D, E, F, G, H, I and J). *CYP17a* mRNA levels were significantly increased in a dose-dependent manner (Fig. 6B), whereas mRNA levels of *LHR, COND2* and *ESR1* were significantly increased even at a low dose (3.5 mg/kg) (Fig. 6C, D and F).

4. Discussion

Many studies have reported that consuming *S. officinalis* in traditional medicine has no side effects (Ghorbani and Esmaeilizadeh, 2017). In the present study, we evaluated the beneficial and/or potentially negative effects of different concentrations of sage extract on rat female reproduction. We found that sage extract had no effect on steroid hormone release when used at low doses (3.5 and 15 mg/kg), as evidenced by the fact that the levels of progesterone, estradiol, and testosterone did not change with treatment. However, at the higher dose (60 mg/kg), estradiol significantly increased. Similarly, the mRNA levels of different genes involved in folliculogenesis and steroidogenesis significantly increased with the high dose of *S. officinalis*. Interestingly, the number of growing follicles significantly increased, whereas the number of abnormal follicles decreased. Further research also revealed that *S. officinalis* promoted growth (Dadras et al., 2020). These effects may be related to the presence of several compounds in this plant. GC–MS analysis revealed that the dominant constituents (>1%) were 1,8-cineole (47.40%), 13-epimanool, (6.45%), 4,4 dimethylandrost-5-ene (5.90%), caryophyllene (5.11%), camphene (4.88%), α-pinene (4.40%), camphor (4.32), β-pinene (2.94%), and ledol (2.32%). No previous studies have reported the effects of these molecules on ovarian function. A previous study showed that the major component, 1,8-cineole, did not cause any toxicities or deaths among rats receiving repeated injections of doses up to 1000 mg/kg for 50 days (Caldas et al., 2016). Sage extract is widely used in the pharmaceutical and cosmetics industries since it has a variety of antitumor (Murata et al., 2013), hepatoprotective (Santos et al., 2001), and anti-inflammatory (Bastos et al., 2011) properties. Diterpenoids represented the second major

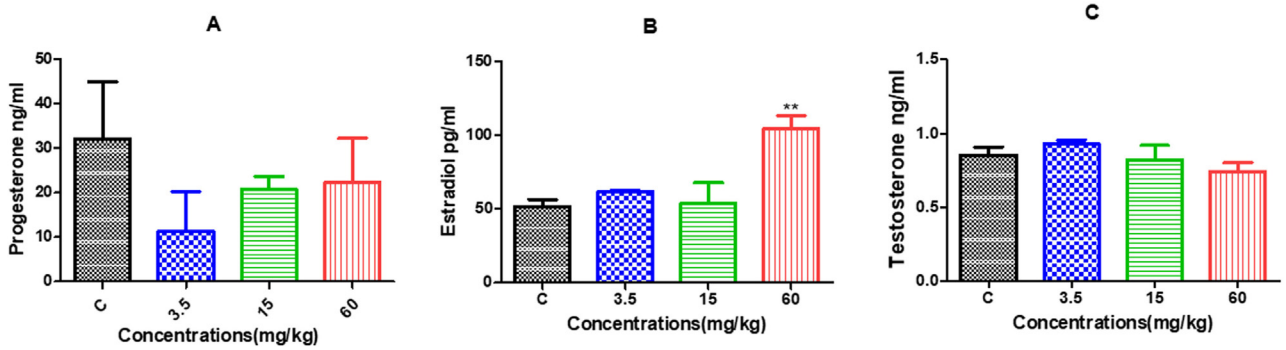

Fig. 2. Serum hormone levels of female rats exposed to sage extracts compared to the control group. Compared to those in the control group, the levels of progesterone (pg/mL) (A), estradiol (pg/mL) (B), and testosterone (pg/mL) (C) in the 3.5 and 15 mg/kg/day groups did not change significantly. However, serum estradiol levels (ng/mL) significantly increased in females treated with high-dose sage extract (60 mg/kg/day) compared to those in the control group. Data are presented as mean ± SEM of triplicate measurements of plasma samples from at least 3 animals from each group.

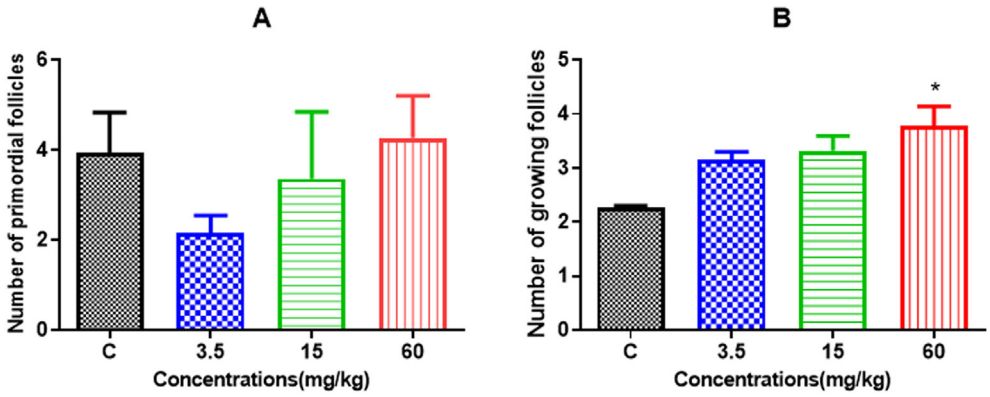

Fig. 3. Effects of different doses of sage extract on the number of primordial (A) or growing follicles (B). All results show the mean ± S.E.M. of three sectioned ovaries for each group, each performed in triplicate. *p < 0.05 compared to control.

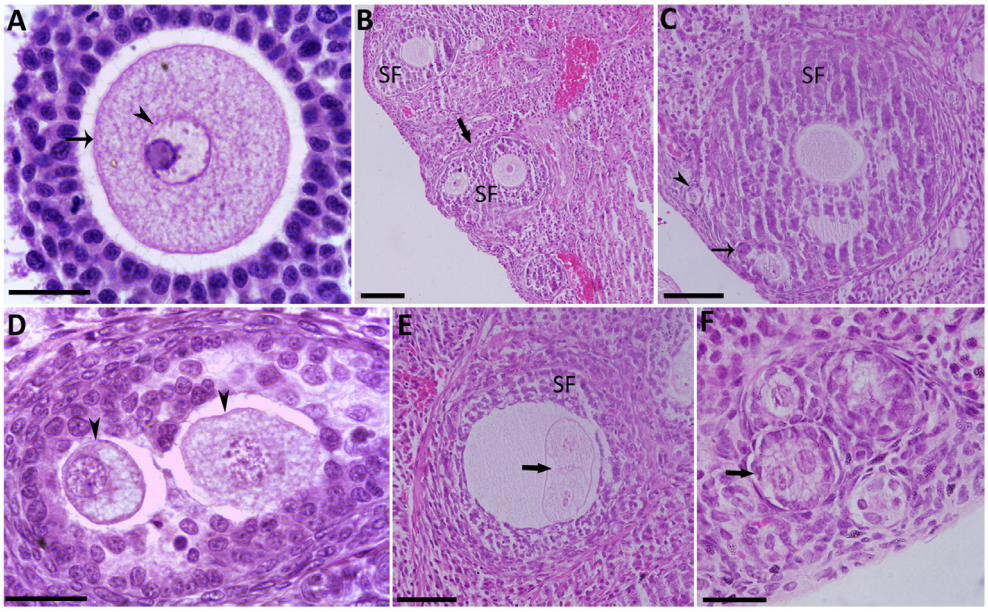

Fig. 4. Ovarian tissue from the different groups showing abnormal follicles by H&E staining. (A) A typical normal secondary growing follicle. (B) Two secondary follicles in the process of merging (large arrow). (C) A secondary follicle merged with a primordial follicle (arrow) and a merging primordial follicle (arrowhead). (D) A secondary follicle containing two oocytes (arrowheads). (E) A secondary follicle with a large oocyte containing two merging oocytes. This is probably a result of two other primordial follicles after their fusion with the secondary follicle. (F) Primordial follicle with a binucleate oocyte (large arrow). SF: secondary follicle; Scale bar = 200 μm.

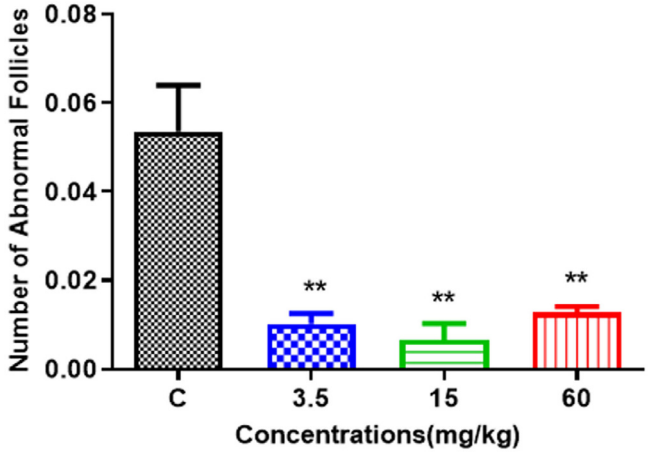

Fig. 5. The number of abnormal follicles decreased significantly in the ovaries of females in the treatment groups (3.5, 15 or 60 mg/kg) compared to the control group. All results show the mean ± S.E.M. of three sectioned ovaries for each group, each performed in triplicate. *p < 0.005 compared to the control group.

component of the sage plant, and 13-epimanool and carnosol have various pharmacological properties, including antiproliferative activity due to their cytotoxic effects (Ruan et al., 2017). Generally, the effects of these compounds on the female reproductive system are not well known, but a few studies have indicated that some of these compounds have adverse effects on female reproduction by reducing the number of atretic follicles and increasing the number of developing follicles (Ruan et al., 2017). However, the effects of the two main components, 1,8-cineole and 13-epimanool, on ovarian function and the mechanisms involved need further exploration.

Since sage extract contains a rich collection of molecules, we hypothesized that high doses of the plant could improve ovarian function through estrogen signaling pathways, estrogen receptors, and ovarian steroidogenesis. Previous studies have reported that some molecules in plants, such as *Senecio biafrae* (Asteraceae), control female reproduction through the nervous system and are used widely in traditional medicine to cure female infertility (Lienou et al., 2020). Therefore, we hypothesize that sage extract may stimulate the release of gonadotropin-releasing hormone (GnRH) and

follicle-stimulating hormone (FSH). FSH binding to granulosa cells leads to activation of FSH receptors that are frequently present on their surfaces. This upregulates the transcription of target genes involved in proliferation and differentiation (Escamilla-Hernandez et al., 2008; Hsueh and Rauch, 2012). Specifically, *INSL3, CCND2, IGF-I,* and *GDF* are essential for the proliferation and differentiation of granulosa cells during follicular growth and development (Yenuganti et al., 2016). Our data show that when we used the higher dose (60 mg/kg) of sage extract, the transcription of these genes increased, which promoted the proliferation of granulosa cells. Estradiol increased after treatment with 60 mg/kg sage extract and exerted its effects by binding to nuclear estrogen receptors (ESR1 and ESR2, also known as ERα and Erβ), consistent with an increase in mRNA transcripts of the corresponding genes. Interestingly, the number of growing follicles increased since estradiol is known to play a vital role in maintaining and developing follicles (Shi et al., 2019). Thus, our results are consistent with dozens of studies that have demonstrated that the estradiol/estrogen receptor pathway plays a key role during folliculogenesis (Liu et al., 2017). Moreover, estradiol activity is enhanced through autocrine signaling of the luteinizing hormone receptor (LHR) to maintain granulosa cell differentiation, leading to ovulation and corpus luteum formation (Kessel et al., 1985). This suggests that the LHR is upregulated in the ovaries of female rats treated with a high dose of 60 mg/kg sage extract. We also analyzed the expression of *CYP17a*, which encodes an enzyme involved in producing androgens (Miller, 2002). Our results showed that the mRNA levels of this gene significantly increased in a dose-dependent manner, suggesting its involvement in follicular development since decreased expression has been linked to reduced ovulation (Maganhin et al., 2014).

We also explored the expression of genes involved in autophagy since we know that many natural compounds are involved in multiple signaling pathways (Wang et al., 2017). Autophagy is an important process that supports cell survival and cell death pathways (Kobayashi et al., 2020). Our results showed that the high dose of sage (60 mg/kg) increased the transcription of all autophagy-related genes that we studied, including *ATG5, LC3*

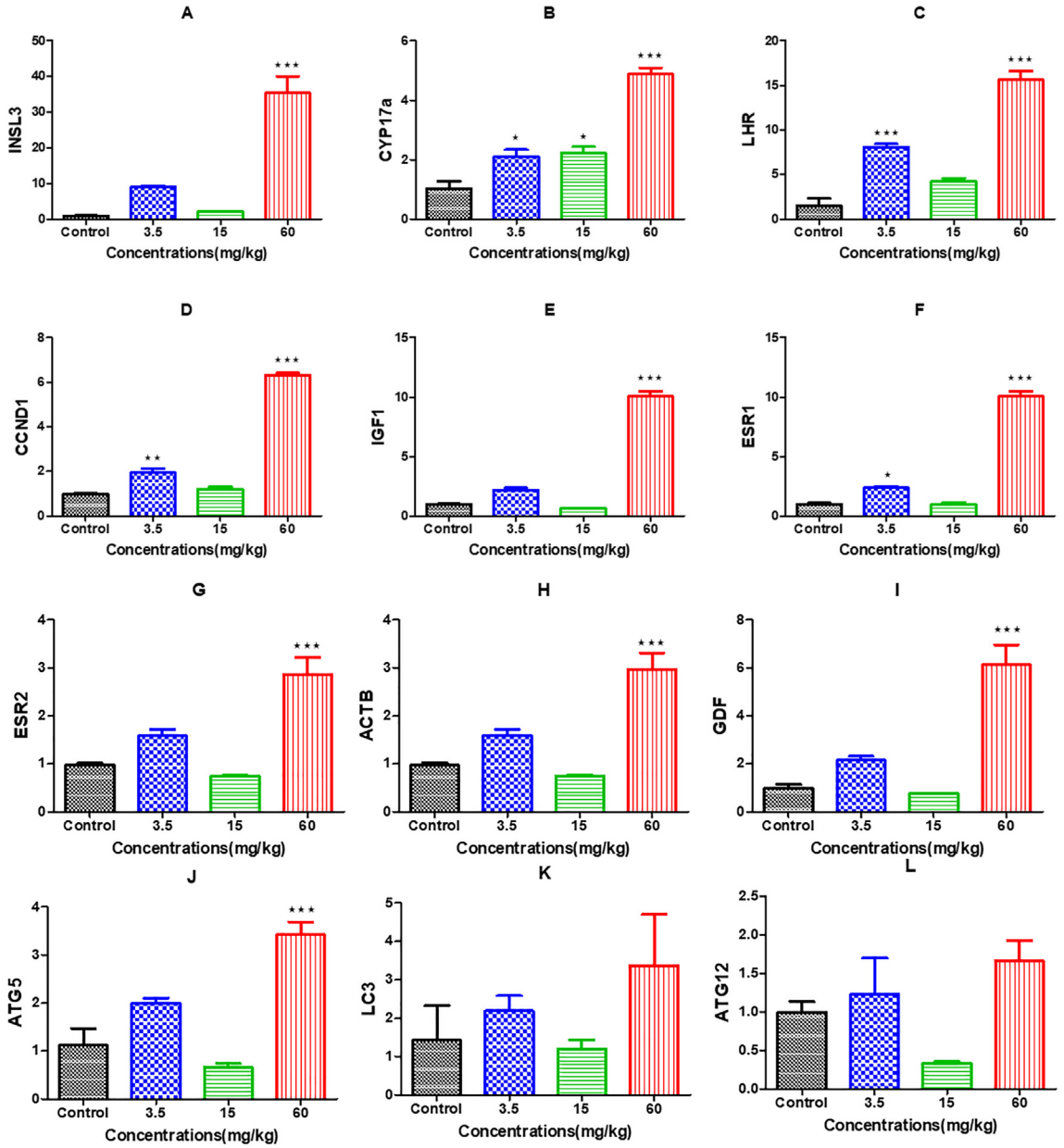

Fig. 6. mRNA expression levels of different genes in the ovaries of rats in the treatment groups compared to the control group.

and *ATG12*. However, the increase was only significant for *ATG5*, which is a key component in autophagy, and its expression is upregulated after DNA damage (Ye et al., 2018). We hypothesized that sage extract induced the expression of *ATG5* to reduce ovarian pathogenesis by activating apoptotic signaling pathways. Recent evidence demonstrated that autophagy inhibition in bovine thecal cells induced the expression of cytochrome CYP17a, contributing to pathogenesis in polycystic ovarian syndrome (PCOS) (Kobayashi et al., 2020). Interestingly, abnormal follicles were sensitive to the effects of sage extract and were reduced in the ovary. Notably, the presence of more abnormal follicles is linked to reduced fertility (Perez-Sanz et al., 2013; Harrath et al., 2017). Additional studies are required to explore the precise molecular mechanisms that regulate the formation of abnormal follicles and to identify methods to reduce them.

One of the limitations of this study was that the estrogen activity could be attributed to the various flavonoid constituents present in sage plants (Michel et al., 2013; Sirotkin and Harrath, 2014; Zingue et al., 2016). These molecules are well-known phytoestrogens that are chemically analogous to mammalian 17β-estradiol and therefore match the binding domain of estrogen receptors. These factors could potentially affect all processes regulated by endogenous estrogens (Sirotkin and Harrath, 2014; Zingue et al., 2016). Due to technical problems, we could not proceed with high-performance liquid chromatography with ultraviolet detection (HPLC-UV) to determine the quantity of flavonoids present in our sage extracts. However, recent evidence has shown that *S. officinalis* extracts contain a total flavonoid content of 11.29 mg QE/mL (Gligor et al., 2020). This suggests that these phytoestrogens can bind to ER1 and/or ER2 in granulosa cells and promote cellular proliferation and cytodifferentiation (Rietjens et al., 2013) by activating proliferative and steroidogenic pathways.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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