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Effect of Different Doses of Volatile Oil Extract of Rosemary in Suckling Rats on Some Physiological Parameters and Pups

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ABSTRACT

Background: The current study was carried out in the animal house-College of the Veterinary Medicine/University of Mosul-Iraq. The study aimed to evaluate the effects of volatile oil extract of rosemary on some physiological and reproductive parameters of suckling female rats and their pups.

Methods: 24 suckling female rats were used and have been distributed randomly to four groups 6 suckling female rats for each group. The first group was considered a control group that was administered only with corn oil orally while the other groups were treated orally with volatile oil extract of rosemary 250,500, 1000 mg/kg body weight for 21 days (time of suckling).

Result: Results revealed that the treatment of suckling female rats with volatile oil extract of rosemary caused a significant P<0.05 decrease in body weight of suckling female and its pups in 7,14,21 days of suckling time and an increase significantly P<0.05 the mortality of suckling female and its pups, as well as increase the growth factor of pups(days) significantly which include (open of ears, obtained of hair, obtain of teeth and opened of the eyes), compared with the control group. Doses of volatile oils extract of rosemary (250, 500, 1000 mg/kg b.w.) caused a significant P<0.05 increase of testicular descent and primary preputial separation of male suckling pups (days) and a decrease significantly P<0.05 Ano-gential distance (AGD)/mm, as well as increased vaginal opening significantly P<0.05 of female suckling pups compared with the control group. There was a significant decrease P<0.05 in each of estrogen, prolactin, FSH and LH in all doses of rosemary oil used in the study, while there was no significant difference in progesterone copared to the control group in suckling females.

Key words: Albino rats, Physiological changes, Rosemary oil, Volitale oil.

INTRODUCTION

Medicinal plants are a group of plants having in their parts chemical constituents which are active in curing ailments (Chomchalow 2000), or they could be defined as those plants which are used for healthcare purposes in both allopathic and traditional medicine systems and covers a wide range of species used including condiments, food, aromatic and cosmetics (Smith-Hall *et al*., 2012).

Many studies have shown the preventive and curative benefits of herbs and medicinal plants. Technical progress has led to isolating effective compounds from medicinal plants, studying their toxicity and determining their effective physiological doses in humans and animals (Akram and Nawaz, 2017). Nowadays herbal medicine is widely used for various reasons such as appropriate and sustainable efficacy, low side effects, low mass and ease of access, *etc*.

In addition, herbal medicine, unlike chemical medicine, contains many substances that are compatible with human nature and protect these substances. The heart and liver, neutralise free radicals and toxins (Nasri and Shirzad, 2013; Mahboubi 2016; Taepongsorat *et al*., 2008). Rosemary is one of the herbs that increases or changes as recommended by the sex hormones (Taepongsorat *et al*., 2008).

Rosemary oil also has healing properties as it acts as an astringent, digestive, diuretic and a source of natural antioxidants (Orhan *et al*., 2012). Some studies have shown that rosemary extract has a positive effect on restoring the testicles and enhancing sperm activity. it was also noted that the use of rosemary oil orally increases fertility and its use in high concentrations will not lead to adverse results (Zihreh *et al*., 1970).

Rosemary *Rosmarinus officinalis* is an organic evergreen plant with needle-shaped leaves that belongs to the family Lamiaceae (Nieto *et al*., 2018), its fresh or dried leaves are used for decoration and to add flavour to foods. It has many biological functions because it is rich in phenolic compounds such as Cafeique acid, Carnosol and Rosmanol, as well as Rosmarinique acid (Sarac and Uğur, 2007). In addition to the fact that the rosemary plant contains

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flavonoids found in the roots, stems and leaves of its flowers, the antioxidant activity of this plant is due to the fact that it contains the above-mentioned compounds, which are very effective antioxidants and have an important role in inhibiting effective radicals (del Baño, 2004). It also proved its effectiveness as an anti-bacterial, anti-inflammatory, anti-fungal and anti-cancer (Kasim *et al*., 2020). Taking high doses of rosemary oil causes gastrointestinal and renal inflammation and there are few studies on the use of the oil extract of this plant during pregnancy and lactation, so this study aimed to evaluate the effect of volatile oils extract from it purely and evaluate different doses of the oil extract of the rosemary plant ROS on the levels of the sex hormones FSH, LH, Estrogen, Prolactin and some physiological and phenotypic parameters in lactating rat mothers and their offspring.

Materials and Methods

Rosemary oil extraction

Rosemary plants were collected from the gardens of Mosul University and dried in dark conditions at room temperature. The dried leaves were immersed in 1 litre of sterile distilled water in a spherical glass flask using a Clevenger device. Distillation was carried out for 4 hours, 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 sulfate and keeping the oil in an opaque bottle at a 4°C (Jalali-Heravi *et al*., 2011).

Analysis of active compounds using gas chromatography GC/MS

The quantity and quality of active compounds in the oil extracted from the leaves of the rosemary plant were diagnosed and estimated by sending the oil to the research centre at Soran University-Erbil and analyzed using Gas Chromatography-Mass Spectrometry, Agilent 7890B type with a separation column with the following specifications J and W DB-5 ms ultra inert GC column, 30 m, 0.25 m, 0.25 µm. Helium gas was used as a carrier at a flow rate (1 ml/min) (Jalali-Heravi *et al*., 2011).

Experimental animals

24 female albino pregnant rats have been used at age of 10-12 weeks and their weights were 197.35±7.32 g. They have been obtained from the animal house in the college of the Veterinary Medicine/University of Mosul, between September 2021 and Februry 2022. The rats were housed in plastic cages (6 rats percage) with stainless steel cover with dimensions 15*15*30 cm. Rats were kept at standard conditions (temperature was 20-25°C and 12 h light/dark cycle) and fed on a daily basis.

Experimental protocol

Twenty-four pregnant female rats were divided into four groups. Each group included 6 rats. All treatments were given orally daily with corn oil for 21 days as the T1 control group, T2, T3 and T4 treatment with volatile oil of rosemary at concentrations of 250, 500 and 1000 mg/kg mg body weight respectively in a row for 21 days (lactation period).

Results and Discussion

After obtaining the volatile oil extract of rosemary and then analyzing its active compounds, as the gas chromatography technique showed that the volatile oil extract of rosemary consists of many active compounds, the most important of these compounds are alpha terpineol, camphor, 1,8-cineole, beta-pinene, alpha-pinene (Table 1). Another study reported similar chemicals to rosemary oil in our results (Mossa *et al*., 2019) which published that the main materials of rosemary oil are 31.45% 1,8-cineole, 8.8611% borneol, 1.01% alpha-pinene, 8.86% linalool, 7.32% camphor, 3.37% linalyl acetate, 3.32% alpha-terpineol, 3.42% Y-Terpinene and 1.82% Cymene. In addition, our findings regarding the components of rosemary oil were consistent with (Kocak *et al*., 2021). The essential oil product and its ingredients change as a result of growing conditions and environmental factors (Kulak *et al*., 2019).

Treatment with the volatile oil extract of rosemary showed a significant decrease, $P<0.05$, in the average weights of mothers for doses of 500 and 1000 mg/kg of body weight compared with the control at the seventh, fourteenth and twenty-one days of birth, while there were no significant differences between the control group and the dose 250 mg/kg at the seventh day, while the same dose of 250 mg/kg showed a significant decrease comparing with the control group on the fourteenth and twenty-one days. Our study agreed with by Mengiste *et al*. (2018), who indicated that the treatment with rosemary extract led to a decrease in body weight, if the percentage of decrease in body weight was 17.07% for the control group, while the treatment group with rosemary extract was 7.85%.

Treatment of nursing mothers with volatile oils extract at different doses led to a significant decrease ($P<0.05$) in the body weight, the statistical analysis showed that the control group led to a significant increase in body weight at the seventh, fourteenth and twenty-one days comparing with the body weights for the same period at doses 250, 500, 1000 mg/kg and the control group showed a significant decrease in the mortality rate of newborns comparing with the two doses of volatile oil extract of rosemary 500 and 1000 mg/kg, while the dose 250 mg/kg did not show significant differences with the control group, while the same dose showed (250 mg/kg) was significantly reduced when comparing with the dose 1000 mg/kg body weight.

Auricle aperture

The group of volatile oil extracts of rosemary plant 500 and 1000 mg/kg showed a significant increase compared with the control group, while the 250 mg/kg group did not show significant differences when comparing with the control group.

Hair appearance

There was a significant decrease in the appearance of hair in the newborns for the control group compared with the total doses of volatile oil extracts 250, 500 and 1000 mg/kg of body weight.

Teeth eruption

The control group showed a significant decrease in the period from birth until the teeth appearance compared with the two-dose group 250, 1000 mg/kg of body weight, in addition to the 500 mg/kg dose group showed a significant decrease comparing with the 1000 mg/kg group when the statistical comparison is done between them.

Eyes opening

The control group showed a significant decrease in the eyes opening compared with the doses of 500 and 1000 mg/kg and the dose group 250 mg/kg showed significant differences from the control group in the appearance of the eyes opening, while the same dose showed a significant decrease comparing with the dose group 1000 mg/kg.

Statistical analysis showed that treatment with doses of 250,500, 1000 mg/kg body weight of volatile oil extract of rosemary led to a significant increase at $P<0.05$ for the control group compared to the above-mentioned dose groups for the hormones LH, FSH, Estrogen and Prolactin. Also, the totals of the above-mentioned doses showed significant differences between them when comparing them for each of the two hormones estrogen and prolactin. Moreover, the 250 mg/kg dose group showed a significant increase at $P<0.05$ in the levels of LH, FSH and FSH when compared with the 500 and 1000 mg/kg dose groups.

The period from birth until necrosis of the newborn

The two doses of treatment with volatile oil extract of rosemary plant 500 and 1000 mg/kg showed a significant increase when compared with the control group, as well as the 250 mg/kg group, which showed a significant decrease compared with the dose group 500 mg/kg of body weight.

The period from birth until the appearance of the vaginal opening for female newborns

The two groups 250,500 mg/kg showed that there were no significant differences between them, in addition, the two groups 500, 1000 mg/kg did not show significant differences between them and the statistical analysis of the two groups 500, 1000 mg/kg of body weight showed a significant increase when comparing each group with the control group separately.

The period from birth until the separation of the foreskin from the glans penis (Table 2)

The control group showed a significant decrease compared with the two groups, 500 and 1000 mg/kg, at the same time control group did not show significant differences from the 250 mg/kg group. Also, the two groups 250 and 500 mg/kg did not show significant differences between them and the two groups 500 and 1000 mg/kg did not show significant differences between them also, while the 250 mg/kg group showed a significant decrease compared with the dose group 1000 mg/kg of body weight.

Table 1: Active compounds diagnosed in rosemary volatile oil using GM/MS.

RT (min)	Area%	Name	Quality	CAS number
6	0.06	Tricyclene	96	000508-32-7
6.157	0.11	α-Thujene	97	002867-05-2
6.409	12.57	.ALPHA.-PINENE	96	000080-56-8
6.823	0.73	alpha.-Fenchene	97	000471-84-1
6.886	5.44	Camphene	98	000079-92-5
7.903	8.22	beta.-Pinene	97	018172-67-3
8.453	1.28	.beta.-Myrcene	96	000123-35-3
8.941	0.28	l-Phellandrene	95	000099-83-2
9.449	0.07	.alpha.-Terpinene	98	000099-86-5
9.837	4.33	Cymene	97	000527-84-4
10.188	48.49	1,8-Cineole	98	000470-82-6
10.393	0.04	trans-.alpha.-Ocimene	97	006874-10-8
11.237	0.24	.gamma.-Terpinene	96	000099-85-4
13.098	0.07	Linalool	93	000078-70-6
15.058	10.24	Camphor	98	000076-22-2
15.436	0.04	L-MENTHONE	97	010458-14-7
15.572	0.06	Isoborneol	94	000124-76-5
16.033	3.90	BORNEOL L	97	000464-45-9
16.337	0.05	Menthol,	91	015356-70-4
16.521	0.22	4-Terpineol	96	000562-74-3
17.171	1.44	.ALPHA. TERPINEOL	91	000098-55-5
19.514	0.25	Pulegone	98	015932-80-6
21.925	1.08	l-Bornyl acetate	99	005655-61-8
27.455	0.66	trans-Caryophyllene	99	000087-44-5
28.698	0.08	.alpha.-Humulene	96	006753-98-6

The distance between the base of the Penis and the outlet

Each group of volatile oil doses of rosemary extract showed a significant increase compared with the control group and the dose group 250 mg/kg showed a significant decrease when compared with each of the two doses of 500, 1000 mg/kg body weight.

Treatment with rosemary oil extract and at doses (250, 500,1000) mg/kg of body weight led to a significant decrease ($P<0.05$) in (average maternal weights at birth, weights between periods 7,14.21 days, birth weights) and an increase in mortality of Newborn compared to the control group (Table 3, 4), especially at doses of 500,1000 mg/kg of body weight. The reason behind the decrease in weight and increase in newborn deaths is due to an increase in the dose of rosemary oil extract and an increase in the period of administration. Rosemary oil extract also affected many physiological characteristics in male and female newborns (hair appearance, teeth appearance, eye-opening, ear-opening, vaginal opening period, the period of separation of the foreskin from the penis and the outlet and the period of testicle descending in newborns (Table 5), as these traits, showed a significant decrease $p<0.05$, compared to the control group and this decrease increased with increasing the dose of rosemary oil extract and the period of administration. Linjawi (2009), showed that Camphor, which is one of the active compounds of the ROS extract, had negative effects that led to significant structural changes in the normal structure of the uterus of pregnant rats, whose effect was reflected in the implantation of embryos in the endometrium and thus caused the abortion of these animals whenever the dose of rosemary oil was increased.

Abou-Hashem, (2012) noted that treatment with alcoholic extract of rosemary led to a decrease in the body weight of white rats, in which weight loss may be due to a decrease in appetite. 21, 14, 7 days of lactation, so that the decrease in the appetite of the mothers leads to a decrease in their consumption of feed and thus a decrease in the production of milk for the mothers, which in turn leads to the newborns consuming quantities of milk that do not meet their daily needs and thus a decrease in their weights.

Moreover, oral administration of rosemary oil extract reduced the levels of estrogen, FSH, LH and prolactin in pregnant mothers compared to the control group, which decreased by increasing the dose of this extract, especially FSH, LH at the doses of 500, 1000 mg/kg body weight and increased at the dose of 250 mg/kg. These results were similar to what was found by Nusier *et al*. (2007), that the aqueous extract of rosemary leaves had a negative effect on the fertility of rats, as it reduced the number of primary, secondary and spermatogenic germ cells with fibroblastsm, in addition, treatment with high doses increased the number of aborted fetuses, a significant decrease in the level of prolactin hormone in nursing mothers at day (21) and thus reduced the fertility of female rats and thus affected the level of male androgen (Table 6).

Table 2: The effect of different doses (250, 500 and 1000 mg/kg) of body weight of rosemary volatile oil on the appearance of some phenotypic characteristics of newborns.

Doses of volatile oil extracted rosemary mg/kg	The period from birth until the testicles descend into the scrotum (day)	The period from birth until the appearance of the vaginal opening for female newborns (day)	The period from birth to the beginning of the separation of the foreskin from the glans penis (day)	Distance between from rod base and outlet hole (mm)
Control	24.6±0.51a	32.6±0.68a	43.8±1.07a	13.29±0.31a
250	25.8±0.49ab	33.8±0.8ab	45.8±1.02ab	11.28±0.42b
500	27.6±0.40c	35.2±0.66bc	48.4±0.93bc	9.81±0.40c
1000	27.2±0.73bc	36.4±0.24c	50.4±1.63c	8.92±0.25c

Damasco and Lemonica (1999) discovered that rats given 260 mg/kg of ethanolic rosemary extract on days 1 and 4 of pregnancy had a significant increase in abnormal fetuses. During the same time period, a higher dose of 1040 mg/kg rosemary resulted in reducing the amount of blastocysts present in the uterus and since chromosomal detection techniques are important factors in both human and animal abortions, the genotoxic and mutagenic effects of rosemary oil could be responsible for the toxic effect on the fetus in mice, as a previous study showed that giving rosemary oil in different doses (300, 1000, (300, 1000) 2000 mg/kg significantly increased DNA damage in rat cells (Maistro *et al*., 2010). Another research team showed that rosemary oil is toxic depending on the dose (Gokturk *et al*., 2020). Using *R. officinalis* rosemary oil at a dose of 20 liters for 24 hours or 10 liters after 48 hours led to 100% insect death.

In a study conducted by Kasim *et al*. (2020), mentioned the aqueous extract of rosemary leaves at a concentration of 10 mg/kg induced a decrease in the level of testosterone hormone in the blood of male rats induced by hyperthyroidism. Pine α-pinene, β-pinenβ, p-cymene and 1,8-Cineole are the natural active compounds found in rosemary oil extract, which have different biological and

Table 3: Effect of the volatile oil of rosemary 250, 500 and 1000 mg/kg body weight on the weight of lactating mothers at birth, 7, 14 and 21 days of suckling.

Doses of volatile oil extract of rosemary mg/kg	Mother's weight of birth (gm)	Mother's weight on the7th day of birth (gm)	Mother's weight on the 14th day at birth (gm)	Mother's weight at the twenty-one day of birth (gm)
Control	201.83±4.36 a	215.33±4.63a	221.50±8.07a	209.17±4.76a
250	203.67±5.05a	194.67±8.51ab	170±7.81b	162±10.26b
500	196.33±6.76a	174.17±9.41b	158.33±12.17b	159.33±13.09b
1000	198.83±7.16a	184±8.37b	162.50±7.22b	142.33±4.13b

-Similar letters indicate that there are no significant differences between groups at P<0.05.

-Different letters indicate the presence of significant differences between the treatments at P<0.05.

Table 4: Effect of different doses (250, 500, 1000 mg/kg body weight) of rosemary volatile oil on birth weight, birth weight at day 7, 14, 21 (weaning) and the total mortality rate for newborns at weaning.

Doses of volatile oil extract of rosemary mg/kg	Birth weight (gm)	Body weight at 7th day (gm)	Body weight on the 14th day of birth (gm)	Body weight on the 21st day of birth (gm)	The total mortalit rate for newborns at weaning
Control	6.32±0.08a	13.98±0.38a	24.45±0.56a	33.45±0.81a	0.50±0.22a
250	6.0±0.08a	10.92±0.33b	18.37±0.57b	23.37±0.84b	2±0.73ab
500	6.19±0.08a	9.86±0.27c	16.52±0.51c	18.62±0.46c	3.67±0.80bc
1000	6.13±0.84a	7.86±0.20d	13.12±0.47d	20.82±0.95c	4.67±0.61c

Table 5: Effect of different doses (250, 500 and 1000 mg/kg) of body weight rosemary volatile oil on the emergence of growth factors, including (aperture of the ear appearance of hair, appearance of the teeth, opening of the eyes).

Doses of volatile oil extract of rosemary mg/kg	The appearance of the auricle aperture (day)	Newborn hair appearing (day)	Newborn teeth appearing (day)	The appearance of the eye-opening for newborns (day)
Control	2.56±0.098a	6.14±0.13a	10.27±0.18a	14.25±0.17a
250	2.40±0.077a	6.90±0.09a	10.21±0.21a	14.72±0.30ab
500	2.50±0.073a	6.62±0.08b	11.68±0.13b	14.97±0.10b
1000	2.53±0.083a	7.98±0.11c	12.54±0.19c	16.03±0.21c

Table 6: Effect of different doses (250, 500 and 1000) mg/kg body weight of rosemary volatile oil on female sex hormones (FSH, LH, prolactin, estrogen and progesterone).

Doses of volatile oil extract of rosemary mg/kg	Estrogen	Progesterone	LH	FSH	Prolactin
Control	1.36±0.10a	0.33±0.03a	1.14±0.07a	1.35±0.09a	2.89±0.10a
250	0.49±0.02b	0.26±0.03a	0.81±0.06c	0.65±0.07C	1.62±0.17b
500	0.3±0.02b	0.31±0.03b	0.28±0.02a	0.48±0.06b	2±0.11b
1000	0.51±0.05b	0.29±0.03a	0.29±0.02b	0.24±0.02b	1.72±0.12b

pharmacological properties as fungicides, antiviral, antibacterial, proliferative, anticoagulant, anti-parasitic and anti-diabetic agents, but the biological activity of these compounds keep for a short time and at low doses because they are ionized, metabolized and eliminated from the body rapidly due to their volatile nature (Cai *et al*., 2021; Salehi *et al*., 2019; Balahbib *et al*., 2021).

Induction of apoptosis is the mechanism of action of 1,8-cineole, which has been shown to inhibit cell proliferation in a dose-dependent manner. As a result, the toxic effect observed in the current study can be attributed to this component. high doses of cineole altered the ultrastructure of the mitochondria and endoplasmic reticulum, as well as the histological structure of the liver and kidneys of mice (Xu *et al*., 2014). Another study found the reproductive toxicity of high doses of cineole, which decreased the amount of corpus luteum, implantation sites and fetal mass in pregnant rats (preimplantation) and increased the number of stillborn fetuses in rats (Caldas *et al*., 2016).

Long-term use of rosemary oil (Rosemary officinalis) reduces the number of follicles and the corpus luteum and estrogen is necessary for follicle development and growth Motaghi *et al*., 2021) and promotes treatment with estrogen divides granulosa cells and increases the weight of the ovaries. The occurrence of such pathological lesions may be due to an increase in the types of active free radicals as a result of giving high doses of rosemary oil extract, as the researchers showed that giving rosemary causes a significant increase in the active oxygen species, which in turn leads to necrotic cellular death (Pérez-Sánchez *et al*., 2019), that is, high doses of rosemary oil caused toxicity and pathological and functional damage in rat ovaries and mammary glands and these changes may be related to hormonal changes.

Conclusion

The current study concludes that high doses of rosemary plant oil extract (250, 500, 1000 mg/kg BW) had toxic effects on some physiological and hormonal characteristics, as well as the fertility of lactating rats and their offspring. As a result, the above-mentioned high doses of rosemary oil can be considered anti-fertility. As a result, after scientific testing, it is possible to take rosemary oil in doses less than 250 mg/kg BW.

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Conflict of interest

All authors declare that they have no conflicts of interest.

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