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ROSE GERANIUM ESSENTIAL OIL RESTORATIVE POTENTIAL ON LEAD ACETATE INDUCED OVARY TOXICITY USING A MOUSE MODEL

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Abstract

Lead is known to cause tissue damage, folliculogenesis dysfunction, and increased follicular atresia. Rose geranium essential oil can be used for therapeutic purposes due to its anti-inflammatory and antioxidant properties. The present study aimed to evaluate the reparative potential of rose geranium (*Pelargonium graveolens* L'Hér) essential oil on the ovarian structure affected by low-dose lead exposure using a mice model. Adult BALB/c mice (n = 18) were divided into three groups: Group I, received physiological saline solution, while groups II and III were administered a single dose of lead acetate. Seven days after lead administration, group III received 300 µl/kg/day of rose geranium essential oil. Histological samples from the group (II) treated with 8 mg/kg of lead acetate showed altered ovarian structure. In contrast, those from group (III), treated with 300 µl/kg/day of geranium essential oil, showed improved ovarian structure. Group (II) showed the highest incidence of follicle atresia compared to the other two groups. Our research demonstrated that rose geranium essential oil had positive effect on the histological structure of the ovary, which had been altered by a low single dose of lead.

Introduction

Lead (Pb) is a heavy, soft, and silvery-white metal that can be a source of public health problems due to its widespread use in various applications (MASSANYI et al. 2020, UNDARYATI et al. 2020). This metal has a high level

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of toxicity that can occur without causing immediate symptoms upon exposure (UNDARYATI et al. 2020).

Lead is known to have adverse effects on the male and female reproductive systems. In women, it can cause reproductive system disorders, including infertility (KUMAR 2018). Experimental studies have reported that lead exposure can produce tissue alterations, edema, necrosis, and denudation of oocytes within ovarian follicles. In addition, it can cause dysfunction in folliculogenesis and increased follicular atresia at all stages of development (DHIR and DHAND 2010, DUMITRESCU et al. 2015, TAUPEAU et al. 2001, UCHEWA et al. 2019, WASEEM et al. 2014).

The effects of certain essential oils are being studied for their potential in the treatment of reproductive disorders. For example, spearmint (*Mentha crispata* L.) essential oil has been shown to reduce follicular atresia in rats with polycystic ovary syndrome (SADEGHI et al. 2017), while chamomile (*Matricaria chamomilla* L.) has been found to restore ovarian structure and increase the number of dominant follicles (ZANGENEH et al. 2010). Rose geranium *(Pelargonium graveolens* L'Hér), a plant that is primarily used by perfumers, belongs to the perennial Geraniaceae family, and its essential oil is known for its regenerative properties (BOUKHATEM et al. 2011). Geranium essential oil has a wide range of benefits. It is a natural relaxant and sedative for the nervous system, and a treatment for bronchitis, laryngitis, and menopausal problems. It is also effective in the treatment of eczema, athlete's foot, and respiratory tract health (RAJESWARA RAO 2013), and is additionally endowed with antimicrobial and antifungal properties (VERMA et al. 2016). This essential oil also exhibits anti-inflammatory and antioxidant properties, suggesting therapeutic potential (BOUKHATEM et al. 2013, SAID et al. 2022).

To our knowledge, no study has investigated the reparative effects of rose geranium essential oil on low-dose lead-induced ovarian toxicity. The aim of the present study was to evaluate the reparative potential of rose geranium essential oil on the ovarian structure affected by low-dose lead exposure, focusing on the overall structure and incidence of growing follicle atresia.

Materials and Methods

Mouse model and ethics statement

Eighteen 8-weeks-old BALB/c mice, with an average weight of 28.3 ±0.37 g, were used in this study. The animals were obtained from the small animal laboratory of the Institut Pasteur d'Algérie and housed in the animal facility of the faculty. Mice had *ad libitum* access to water and food on a 12-hour light / 12-hour dark cycle. Throughout the experiment, temperature was maintained at 20 ±0.17°C, with humidity level of 60 ±0.04%. All procedures were conducted in compliance with Algerian legislation (Law number 95-322/195) concerning the protection of animals intended for experimentation and other scientific purposes. All animals were treated humanely and in accordance with the ARRIVE guidelines and approved by the faculty's scientific committee.

Chemicals and essential oil

Lead acetate trihydrate Pb $(CH_3COO)_2$ was obtained from the Faculty Chemical Store, while rose geranium essential oil (*Pelargonium graveolens* L'Hér. 1789) was extracted via steam distillation and provided by Yakouren Biocare, Algeria. Based on several studies, the main constituents of geranium essential oil were citronellol, geraniol, linalool, citronellyl formate, geranyl formate and isomenthone (VERMA et al. 2016, BOUKHRIS et al. 2013, RAJESWARA RAO 2013, BOUKHATEM et al. 2010).

Experimental design

After 1 week of acclimatization, the estrous cycle of mice was synchronized using Whitten effect (ZAKARIA and SUKARDI 2019), and the animals were randomly assigned to three groups. They were weighed daily to monitor weight trends, and all treatments were administered intraperitoneally. The experiment was conducted for 28 days as follow:

Group I: Received physiological saline solution from the 7^{th} to the 28^{th} day.

Group II: Received a single dose of lead acetate on the first day.

Group III: Received a single dose of lead acetate (8 mg/kg) on the first day, followed by a daily dose (300 µl/kg) of geranium essential oil starting from de the 7^{th} day. The dose of essential oil was chosen based on the study of BOUKHATEM et al. (2013).

Histological specimen preparation

At the end of the experimental procedure, all animals were euthanized, and their ovaries were extracted, degreased, weighed, and fixed in Bouin's fluid for 24 hours. Specimens were then embedded in paraffin blocks and, sections of 5 µm thickness were cut and stained with hematoxylin-eosin (CARSON and HLADIK 2009). Histological observations were performed using a Leica DM/LS light microscope at magnifications of ×100 or ×400.

Atretic growing follicle count

For each sample, one section was taken every 10 µm, resulting in 5 slides for each ovary. To evaluate atretic growing follicles, identification was conducted at ×100 or ×400 magnification. A primary follicle was noted if it contained an oocyte surrounded by a single layer of cuboid granulosa cells, while a secondary follicle was identified by the presence of, at least, two layers of cuboid granulosa cells surrounding the oocyte, without an antrum. An antral follicle was considered if it exhibited multiple layers of cuboidal granulosa cells with an antrum (Kim et al. 2022).

We evaluated growing follicle atresia based on the method described by Uslu et al (2017). In summary, for primary and secondary follicles, we assessed the integrity of the oocyte and counted the number of pycnotic nuclei for each follicle. An antral follicle was considered atretic if it exhibited a fragmented oocyte (FO) or if it was deformed (FD). Atretic follicles were counted using a Leica DM/LS light microscope at ×400 magnification for primary and secondary follicles, and at ×100 magnification for antral follicles.

Statistical analysis

The total number of atretic follicles was counted and compared between the different groups. Additionally, for antral follicles, each category of fragmented oocytes (FO) and deformed follicles (FD) was counted and compared separately. Statistical analysis was conducted using analysis of variance (ANOVA) followed by Tukey's post hoc test (Lafaye et al. 2014). The statistical software RStudio software version 2023.06.0-421 was used for data analysis.

Results

No mortality or behavioral changes were observed in any of the animals throughout the entire experimental period. Additionally, there were no changes in body weight or absolute and relative ovary weights in any of the groups.

Histological description

Histological examination of samples from control group (group I) revealed a normal ovarian structure without detectable alterations. Various stages of follicle development and corpus luteum were observed in the cortex, along with well-organized connective tissue and blood vessels in the stroma (Figure 1*a*). The oocytes appeared intact, and the granulosa cells were well-organized (Figure 1*b*). In contrast, samples from group (II), treated with 8 mg/kg of lead acetate, showed altered ovarian structure. Aeras of optical vacuum within the ovarian tissue were noted, along with advanced atresia of primary and secondary follicles, and diffuse edema. Denudation and deformation of the oocytes, as well as disorganization of the follicular cells at all stages of follicular development, were observed (Figure. 1*c*). Particularly affected were follicles, showing disorganized follicular layers and cells lacking adhesions (Figure 1*d*). However, in group (III), treated with 300 µl/kg of geranium essential oil, an improved ovarian structure was observed. Fewer areas of optical vacuum were noted under the optical microscope, and there was no edema (Figure 1*e*). Developing follicles appeared perfectly structured (Figure 1*f*).

Atretic growing follicle count

Primary and secondary follicle atresia was more pronounced in the group (II), treated with 8 mg/kg of lead acetate, than in the other two groups. There was a significant difference ($P < 0.01$) in the number of atretic primary follicles and a highly significant difference ($P < 0.001$) in the number of atretic secondary follicles (Table 1).

Group (II), treated with 8 mg/kg of lead acetate, exhibited the highest number of atretic antral follicles ($P < 0.05$). Specifically, the number of deformed antral follicles (DF) was higher in the group (II) treated with lead ($P < 0.05$) compared to the other two groups. However, the number of antral follicles with a fragmented oocyte (FO) remained unchanged across the different groups (Table 2).

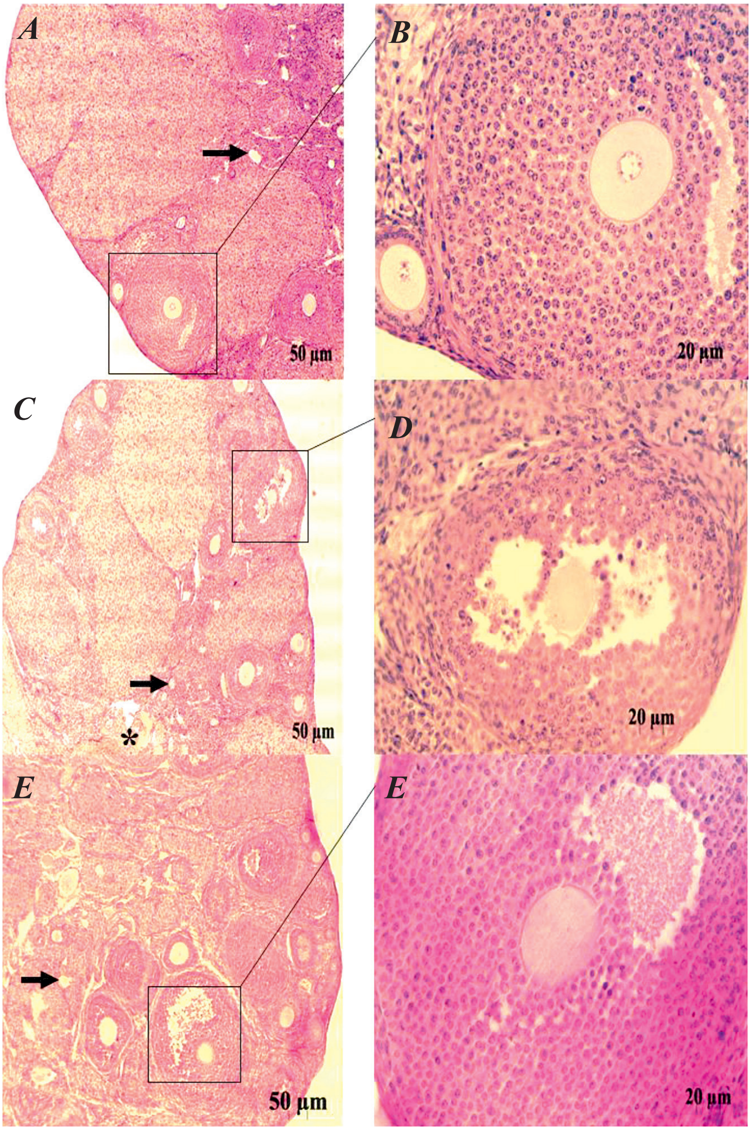

Fig. 1. A photomicrograph showing the ovarian histology from experimental groups stained with hematoxylin-eosin. *A* – control group ovary (I) at ×100 magnification. Unaltered tissue and follicles at different stages of development can be noted; *B* – detail of control ovary at ×400 magnification showing antral follicle with oocyte surrounded by perfectly arranged granulosa cells; *C* – ovary of group (II) treated with 8 mg/kg lead acetate at ×100 magnification. Diffuse edema is indicated by asterisk (*); *D* – antral follicle from group (II) at ×400 magnification exhibiting oocyte denudation and disorganized granulosa cells; *E* – ovary from group (III) treated with 300 µl/kg/day of geranium essential oil at ×100 magnification. Improved ovarian structure and follicles at different stages of development are observed; *F* – detail of antral follicle from group (III) at ×400 magnification showing improvement of follicular structure with no oocyte denudation, well-structured granulosa cells. Arrow indicates atresia of primary and secondary follicles in all groups

Table 1

Primary and secondary atretic follicle count

Groups	I	II	III
APF	6.83 ±2.23	20.7 ±5.68**	6.33 ±2.98
ASF	4.67 ± 0.51	7.68 ±0.51****	3.83 ±1.33

Explanations: The data are expressed as mean ± standard deviation. Groups (n = 6): control (I), 8 mg/kg of LA (II), 8 mg/kg of LA and 300 μl/kg essential oil (III). (APF) atretic primary follicles, (ASF) atretic secondary follicles. An asterisk (*) indicates a significant difference: group (I) and (III) versus group (II): $^{**}P < 0.01$, $^{****}P < 0.0001$

Table 2

Antral atretic follicle count

Groups	I	II	III
ATR	0.5 ±0.9	1.07 ±1.23*	0.36 ±0.72
FO	0.3 ±0.59	0.36 ±0.8	0.2 ±0.55
DF	0.2 ±0.48	0.7 ±0.98*	0.16 ±0.48

Explanations: the data are expressed as mean ± standard deviation. Groups (n = 6): control (I), 8 mg/kg of LA (II), 8 mg/kg of LA and 300 μl/kg essential oil (III). (ATR) total of atretic antral follicles, (FO) antral follicles with fragmented oocyte, (DF) deformed antral follicles. The asterisk (*) indicates a significant difference: group (I) and (III) versus group (II): $^{*}P < 0.05$

Discussion

Our results indicated that a low-dose administration of lead did not induce any change in body weight or ovarian weight in mice as reported by previous studies (TAUPEAU et al. 2001).

The literature reports that heavy metals have adverse effects on reproductive function, which can lead to infertility (BHARDWAJ et al. 2021, MASSANYI et al. 2020, RZYMSKI et al. 2015). Our histological observations showed that lead acetate induced an alteration in ovarian structure, particularly in the follicles, and are consistent with several studies demonstrating that this heavy metal causes damage to the ovary. In fact, at low doses, lead acetate causes hyperemia and degeneration of follicles at all stages of development, and vacuoles may be observed at several sites (DUMITRESCU et al. 2015, JUNAID and CHOWDHURI 1997).

Lead is a reprotoxic agent that accumulates in granulosa cells and induces morphological changes (JUNAID and CHOWDHURI 1997, VYLEGZHANINA et al. 1993). According to USLU et al. (2017), primary and secondary follicle atresia was assessed by the oocyte integrity and the number of pycnotic nuclei, while that of the antral follicles was determined by oocyte integrity and follicle shape. Our results showed thar follicular atresia was higher in the group treated with lead acetate, particularly in the secondary follicles, where the difference was highly significant (Table 1). Additionally, we noted that the number of deformed antral follicles (DF) was higher than in the other groups (Table 2). The majority of ovarian follicles fail to ovulate and degenerate through atresia (MCGEE and HSUEH 2000). However, several studies have reported that lead exposure can cause and exacerbate follicular atresia at all stages of development (QURESHI et al. 2010, TAUPEAU et al. 2001).

A large number of studies have focused on the effects of natural substances on health (AKINYEMI et al. 2018, BELLIK 2022, SAKHTEMAN et al. 2020). However, few studies have examined the effects of plants and their essential oils on female reproductive function (DOMARACHY et al. 2007, PARANDIN and YOUSOFVAND 2019, PARHIZKAR et al. 2016). To our knowledge, the effects of rose geranium essential oil on female reproductive function have never been studied, and very few studies have investigated the role of essential oils in restoring lead-damaged ovarian structure. Nevertheless, it is generally agreed that some of them have therapeutic properties. For example, research on the possible effect of essential oils on reproductive disorders in females indicates that administration of 300 mg/kg of spearmint essential oil to rats with polycystic ovary syndrome significantly reduced follicular atresia and restored ovarian structure (SADEGHI et al. 2017). In the context of fertility enhancement, administration of common fennel extract to healthy mice improved folliculogenesis and reduced the number of atretic follicles at all stages of development (KHAZAEI et al. 2011).

Follicular atresia is a natural event within the ovary, and apoptosis-induced follicular atresia can originate from granulosa cells or oocytes (XUAN and YIXUN 2003). As shown in our results, low-dose lead acetate administration altered the histological structure of the ovary and increased follicular atresia. However, structure and follicular degeneration were reduced by administering rose geranium essential oil. According to OUIES et al. (2020), lead acetate-induced follicular atresia in a pubertal and prepubertal rat model was reduced by *Nigella sativa* administration.

The main compounds of the Algerian geranium cultivar are citronellol and geraniol, natural monoterpenoids endowed with antioxidant and anti-inflammatory properties (ABE et al. 2004, BOUKHATEM et al. 2013, KOBAYASHI et al. 2016, MARUYAMA et al. 2006). The reduction in follicular atresia in group (III) is most probably due to the antioxidant properties of this essential oil. As reported by CAVAR and MAKSIMOVIC (2012), geranium essential oil can be a good source of antioxidants, and a study by BOUKHRIS et al. (2015) reported that the extract and essential oil of rose-scented geranium showed antioxidant activity. In fact, the results of the study suggest that the major components of this oil can inhibit a range of free radicals. The administration of lead acetate has been observed to induce alterations in the antioxidant system, due to the activation of free radical oxidation (OSOWSKI et al. 2023). Therefore, it is imperative to investigate the antioxidant effects of this oil as a potential mechanism of action in attenuating ovarian toxicity induced by low doses of lead.

Conclusion

Our findings revealed that rose geranium (*Pelargonium graveolens* L'Hér) essential oil had a positive effect on the mouse ovary's histological structure, which was altered by a low single dose of lead. It was observed that there were no signs of toxicity, and growing follicle atresia was reduced. Therefore, this study pointed out rose geranium essential oil' restorative potential on lead acetate induced ovary toxicity in a mice model, while further research is needed to investigate the associated mechanisms.

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