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Investigating the Impact of *Launaea angustifolia* Extract on the Reproduction Function of Female Rats

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ABSTRACT

Background: The purpose of this study is to investigate the effects of *Launaea angustifolia* methanol extract on the reproductive organs of female rats through the use of chemical screening.

Methods: A total of twenty-five female rats were separated into five distinct groups to be examined. The group that served as the control did not receive any dose, whereas the group that served as the negative control received DEMSO by oral gavage. Group 10 received 10 mg/kg body weight per day, Group 50 received 50 mg/kg body weight per day and Group 100 received 100 mg/kg body weight per day for a period of two weeks.

Result: Despite the fact that the body weights of female rats rose during the course of this investigation, there were no significant variations between the groups after 14 days of dosing occurred. In the case of female rats, the body weights of the control group were found to be considerably greater ($P<0.05$) in all experimental days, with the exception of day 1 and day 3, when compared to the groups that received 50 and 100 mg. Although there was a discernible rise in the weight of the ovary in the control group in comparison to the group that was exposed to DMSO.

Key words: Extract, Female, Hormonal, *Launaea angustifolia*.

INTRODUCTION

Recently, there has been a growing trend among people from various nations to utilize traditional medicine as a supplementary or alternative approach for addressing common health conditions (Yekta *et al.*, 2023; Jamal, 2023). There are numerous medicinal plants that are used as dietary supplements or biopharmaceuticals instead of manufactured drugs. Nevertheless, a considerable number of these medicinal plants necessitate additional scrutiny regarding their adverse effects and examination of their constituents (Goshtasbi *et al.*, 2023). The characteristics of phytochemical constituents range significantly among various species. The *Launaea* genus, belonging to the Asteraceae family, encompasses a total of 55 to 59 species distributed over several countries in the southern and western regions of the Mediterranean, as well as in North Africa and Southwest Asia.

Launaea comprises a total of forty species that possess medicinal capabilities, including the ability to alleviate stomachache, treat skin illnesses, combat cardiovascular diseases, lower cholesterol levels, fight against cancer, inhibit microbial and fungal growth, reduce inflammation, function as antioxidants, exhibit cytotoxic effects and possess neuropharmacological properties (Marzouk *et al.*, 2021; Michel *et al.*, 2020). Moreover, the anti-diabetic properties of the ethanolic extract of *Launaea nudicaulis* in rodents induced with streptozotocin have been attributed to the flavonoid and phenolic compounds it contains (Kumer *et al.*, 2023). Moreover, the anti-diabetic properties of the ethanolic extract of *Launaea nudicaulis* in rodents induced with streptozotocin have been attributed to the flavonoid and phenolic compounds it contains (Khan *et al.*, 2023), while reducing by the activation of oxidative defense mechanisms, alloxan-induced pathological consequences (Gbadamosi *et al.*, 2020). Moreover, El-Fadaly *et al.* (2023) The researchers documented the capacity of *Launaea mucronata* extract to enhance biochemical indicators of renal function in a chromium-treated rodent model (K2Cr2O7). In particular, both male and female rodents exhibited a protective effect against hepatic enzymes and kidney functions when exposed to *Launaea angustifolia* (Aiman *et al.*, 2023). Despite therapeutic and toxicological studies using *Launaea* sp. extracts, their effects on reproductive physiology in male and female rats have not been studied until now. Therefore, the primary objective of this study is to investigate the hormonal and histological transformations in the reproductive organs of rats after the administration of *Launaea angustifolia* methanol extract.

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MATERIALS AND METHODS

Chemicals

Unless otherwise specified, all chemicals utilized in this undertaking were acquired from Sigma.

Plant collection and extraction

The *Launaea angustifolia* plant was collected during the time from 1st Feb to 1st April 2022, from the Al-Thumamah area (25.25141°N 46.63067°E), Riyadh city. The leaves were removed from the plant, washed and kept in a dark and cleaned place until dried. Then, the leaves were grained until they became powdered. The crushed plant leaves of *Launaea angustifolia* were soaked in methanol for a day. Later, the solution was filtered via the Whatman paper. After maceration, the *Launaea angustifolia* extract was evaporated at a temperature between 50 and 60°C, then the powder was frozen at -20°C until the dosing preparation. Before the trial, one hundred milligrams of the *Launaea angustifolia* extract was dissolved in DMSO to prepare the concentrations (10, 50 and 100 mg/kg of rat) required for dosing (Alhimaidi *et al*., 2022).

Experimental design

female rats in good health were obtained from the animal facility at the Zoology Department of the Science College, King Saud University (KSU). The rats were accustomed to a well-ventilated area with a suitable room temperature of 25±2°C, a regular light and dark schedule of 12 hours each and provided with a standard diet and water. All experimental procedures were conducted in compliance with the guidelines specified by the ethics committee (Approval no: KSU-SE-23-06) and the Institutional Animal Care at KSU. The project was conducted on female rats (25 rats / female), with an average weight 200-240 g and ages between 12 and 15 weeks. Each female of rats was divided separately into five groups (5 rats / group). The G-control was without any dosage, while the G-negative control was treated by oral gavage with DMSO. The G-10, G-50 and G-100 were given 10, 50 and 100 mg/kg bw/day respectively of *Launaea angustifolia* extract for 14 days.

Biochemical analysis

At day 15, Blood was drawn from animal heart in non-heparinized tubes, cooled at ~4°C for overnight, then centrifuged at 1000x g for 15 min to get serum and frozen at -20°C up to hormonal studies. The enzyme-linked immunoassay (ELISA) method was used to estimate all hormone concentrations in serum samples by the following ELISA kits (MOLEQULE-ON company, Auckland, New Zealand): 17 β-estradiol (Cat No. ELI-M-036-96), Progesterone (Cat No. ELI-M-034-96).

Histomorphometric examination

The ovary were collected after the animals were anesthetized with CO_2 and socked in 10% neutral formalin for 48 hours for fixation. The fixed gonads were dehydrated by gradual alcoholic solutions, infiltrated in xylene for 4-6 hr., impeded in paraffin wax cubes, sectioned by microtome, mounted on slides and stained with H&E. The histomorphological and histomorphometric evaluations were examined by a light microscope. Ovarian follicles were counted and classified in all animal groups according to Adelakun *et al.* (2022) with some modifications. Primordial Follicles were identified by the patchy row of squamous cells around the oocyte. Primary and secondary follicles were recognized by the presence of one row and two rows of cuboidal cells respectively. The tertiary follicles were distinguished by the presence of numerous layers and small antrum. Graafian follicles were characterized by a large follicular antrum.

Statistical analysis

All statistics obtained from this experiment were analyzed by the GraphPad Prism (10.1.0) program. The Shapiro-Wilk test demonstrated the normal distribution of our data. The animals body weights were analyzed using two-way ANOVA (day factor × extract factor) and Tukey's multiple comparisons test. Hormone levels, weights of the reproductive organs data were examined using one-way ANOVA, followed by Tukey's test. The outcomes are presented as the average values and standard deviations and any P value less than or equal to 0.05 indicates a significant finding.

RESULTS AND DISCUSSION

Although the body weights of female rats increased during this study, there were no significant differences between groups after 14 days of dosing (Fig 1).

Female rats, the body weights were significantly higher ($P<0.05$) in all experimental days (except day 1 and day 3) in the control group compared to the 50 and 100 mg groups. While there was a significant increase in ovarian weights in the control group compared to the DMSO group (Fig 2).

17 β-estradiol and progesterone levels in all female after the end of the experiment are shown in Fig 3, 4. There were no statistically significant differences between the concentrations of 17 β-estradiol and progesterone in the female groups under study.

Percentages of ovarian follicles in their different stages there are significantly different in treated groups 10 g and 50 g (follicle stage) compared to control group at $P<0.05$ (Fig 5).

The scientifically validated approach to identify pharmaceuticals or herbal medicinal formulations involves conducting safety evaluations on different experimental animals prior to their use in humans. The *Launaea* genus plant is widely recognized for its extensive use in traditional medicine for the treatment of many ailments.

The objective of our inquiry is to examine the impact of *L. angustifolia* extract chemical screening on the reproductive function of female rats. The findings of our study validated that the *L. angustifolia* extract, which contained significant levels of fatty acids, significantly improved

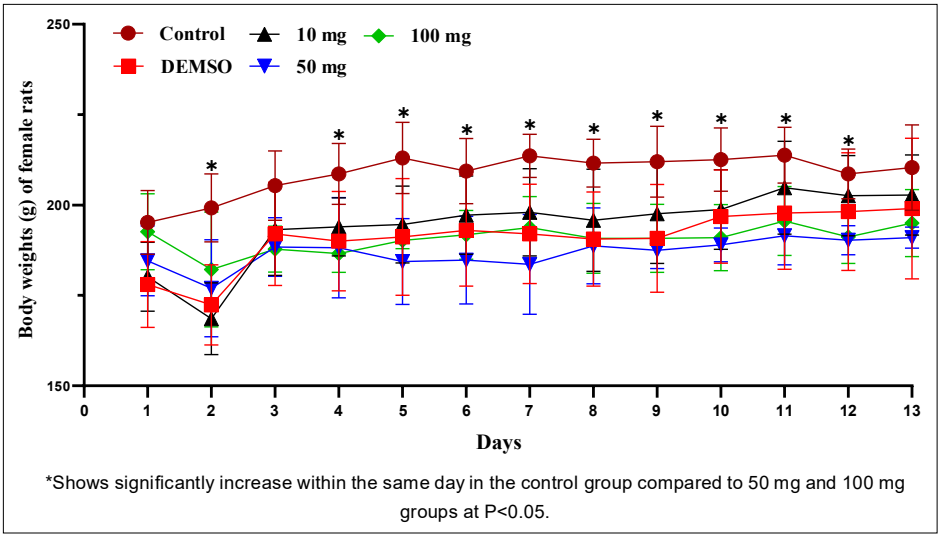


Fig 1: Body weights (g) of female rats during the experimental days.

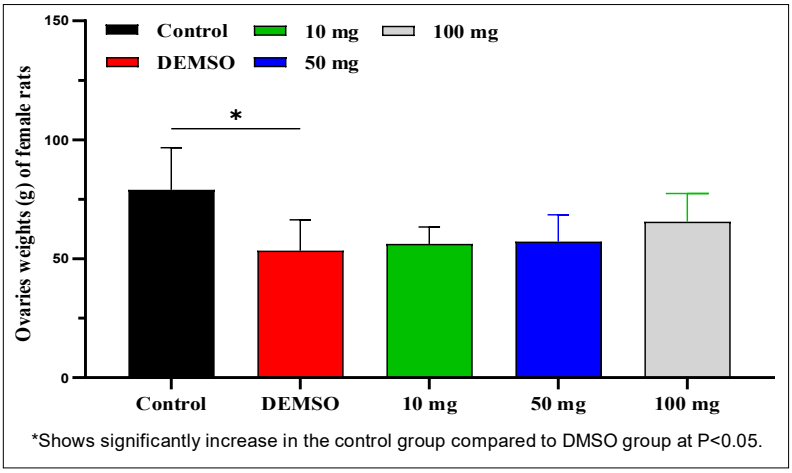


Fig 2: Ovaries weights (mg) of female rats at the end of experiment.

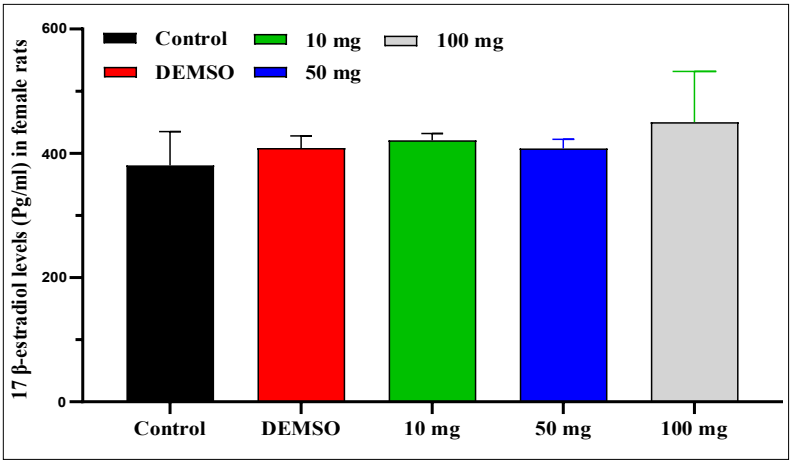
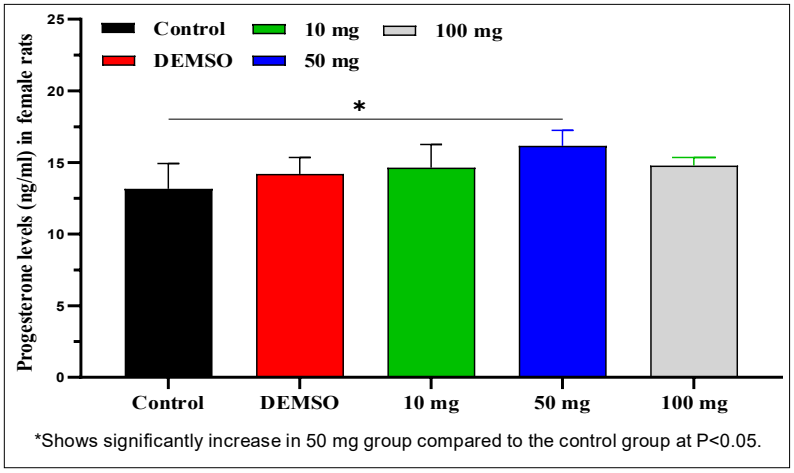
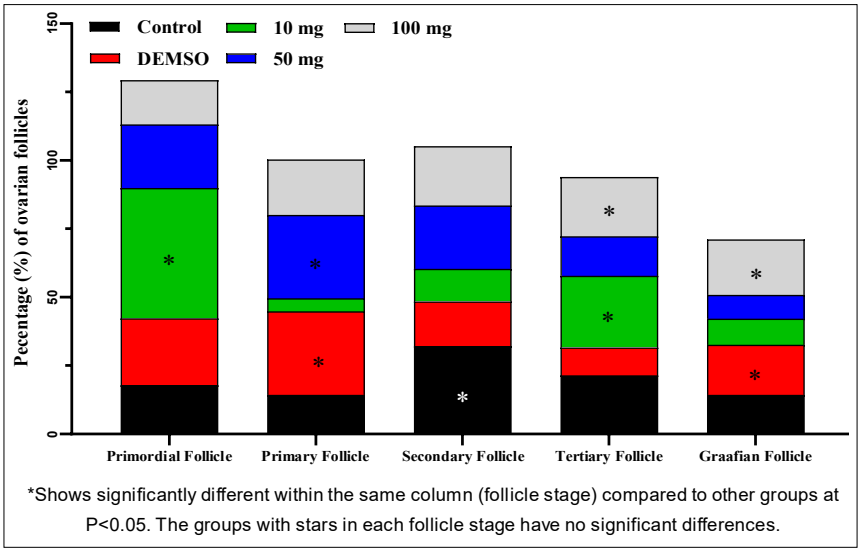


Fig 3: The 17 β-estradiol levels (Pg/ml) in serum male rats at the end of experiment.



*Shows significantly increase in 50 mg group compared to the control group at P<0.05.

Fig 4: The progesterone concentrations (Pg/ml) in serum male rats at the end of experiment.



*Shows significantly different within the same column (follicle stage) compared to other groups at P<0.05. The groups with stars in each follicle stage have no significant differences.

Fig 5: The percentages of ovarian follicles in their different stages in all groups.

reproductive functions in female rats that were subjected to the extraction.

Upon doing a chemical examination on the extract of *L. angustifolia*, it was discovered that the presence of Oleic acids, Linoleic acid, n-Hexadecanoic acid and Octadecanoic acid, along with other phytochemicals, was present in a minor quantity.

A group of researchers who analyzed the chemical makeup of *Launaea resedifolia* and found that it included 19 compounds, the most of which were dominated by dioctyl phthalate oil, with the majority of the chemical linkages being esters, alcohols, ketones and terpenes. Cheriti *et al.* (2006) observed that the oil content of *L. angustifolia* and that of *L. arboresens* were quite similar to one another. This was also the case when comparing the two species.

There is a possibility that the variation is attributable to the geographical dispersion as well as the time of collection. In contrast, the essential oil of *Launaea lanifera*, which was investigated by Benmeddour *et al.* (2015), was distinct from the essential oil of *L. angustifolia* species. According to Benmeddour *et al.* (2015) and Pavia *et al.* (2015), the absence of a distinctive aroma in the *L. lanifera* oil was related to the fact that the plant material utilized in the *L. lanifera* oil extraction process comprises a significant quantity of flowers. Additionally, the plant material does not contain any aromatic chemicals.

CONCLUSION

The findings of this experiment demonstrated that the significant quantity of fatty acids included in the extract of *L. angustifolia* associated with an improvement on the reproductive function of female rats that were exposed to the extract, particularly at high doses. It is possible that the *L. angustifolia* extract, which has been shown to have medicinal and therapeutic benefits, might be a strong candidate for the discovery of further pharmacological qualities.

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Data availability

The data sets used in the current study are available from the corresponding author upon request.

Ethics approval

All experimental procedures were conducted in compliance with the guidelines specified by the ethics committee (Approval no: KSU-SE-23-06) and the Institutional Animal Care at KSU.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflicts of interest

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