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# Effects of Short and Long Term Treatment of *Ballota undulate* on Female Albino Rats Fertility and Pregnancy

Walid Sh. Qazan
Department of Animal Production, Faculty of Agriculture and Science,
Jerash Private University, P.O. Box 311, Jerash 26110, Jordan

**Abstract:** The objectives of this study is to investigate the toxic effects of *Ballota undulata* (300 mg $kg^{-1}$ b.wt.) on the reproductive system after administration to female Sprague-Dawley rats for two time periods 4 and 12 weeks. Forty adult female Sprague-Dawley rats were divided into two treatment and two control groups of 10 rats each. The two treated groups received 15 mg $kg^{-1}$ b.wt. of *Ballota undulata* for two periods of 4 and 12 weeks. Female rats were allowed mating with males after treatment. Several pregnancy parameters were investigated including: total number of pregnant rats, body weight and reproductive organ weight, number of implantation sites, number of resorption sites and number of viable fetuses. The significance of these results was calculated using student's t and Chi-square tests. Exposure to *Ballota undulata* for 4 weeks did not have significant effects on most parameters investigated. However, a slight decrease in the relative ovarian and embryo weights was observed. Administration of *Ballota undulata* for 12 weeks significantly reduced the percentage of pregnancies and the number of implantation sites when compared with controls. In addition, a decrease in ovarian weights and in viable fetuses' number was also observed. Histological sections of ovaries of female treated with *Ballota undulata* were found to have congested blood vessels in cortical and medullary regions. We also noted the arrestment of developing follicles at primary and secondary stages in addition to presence of degenerative areas and hypercellullarity in medulla. Long term treatment with *Ballota undulata* might lead to diversified negative effects on fertility and pregnancy in female rats.

**Key words:** *Ballota undulate*, female rats, fertility, pregnancy, reproductive organs

## INTRODUCTION

The vast majority of modern medications were derived originally from ancient herbal traditions (Yu *et al*., 2006; Lahans, 2007; Ngemenya *et al*., 2006).

Medicinal plants have been used for centuries as remedies for human diseases as they contain components of therapeutic value (Ramoutsaki *et al*., 2002). There are numerous natural plant products which have antifungal, antibacterial and antiprotozoal activities that could be used either systemically or locally (Lee *et al*., 2007). Several plants containing volatile oils, polyphenols and alkaloids as active constituents are utilized as popular folk medicines, while others gained popularity in the form of finished products collectively named phytomedicines (Shanker *et al*., 2007).

The use of medicinal plants for the treatment of diabetes mellitus dates back to the Ebers papyrus, 1550 BC (Bailey and Day, 1989). Even after the discovery and use of insulin and other modern oral hypoglycaemic agents, the search for safer and more effective drugs of plant origin for the treatment of diabetes has continued (Bailey and Day, 1989; Grundy, 2006).

Through the ages, physicians have attempted the treatment of diabetes mellitus with indigenous plant (Leduc *et al*., 2006). Many herbs and plants that exhibit hypoglycemic activity when taken orally have been described (Shane-McWhorter, 2005). Some of these plants have also been pharmacologically proven to have a value in the treatment of diabetes mellitus (Lewis and Elvin-Lewis, 1994).

*Ballota undulata* (Lamiaceae) is a Mediterranean plant but has a more continuous distribution in a wider range of relatively moist microhabitats, up to 800 m above the sea level; it has less than 15 leaves, usually erects and undulates, with white to dark pink flowers(Citoglu *et al*., 2004). Its distribution is affected positively by elevation. *Ballota undulata* prefers low-pH soils, share soil microhabitats with high clay and silt and organic matter. These habitats have low sand content, low pH and relatively high soil moisture (Citoglu *et al*., 2004; Zaghloul, 2003).

The most important constituents of *Ballota undulata* are monoterpenes and sesquiterpenes (Bader *et al*., 2003).

This plant was suggested to exert anti-allergic, antispasmodic, antimicrobial and anti-inflammatory properties (Al-Bakri and Afifi, 2007).

The objectives of this study is to investigate the toxic effects of *Ballota undulata* (300 mg $kg^{-1}$ b.wt.) on the reproductive system after administration to female Sprague-Dawley rats weighting 250-300 g for two time periods 4 and 12 weeks.

## MATERIALS AND METHODS

**Plant material and extraction:** The *Ballota undulate* fruits were collected in September 2006 from bursa. The plant was authenticated by a botanist. The extract was prepared according to WHO protocol CG-04 for preparation of an alcoholic extract (WHO Protocol CG-04, 1983). Briefly 100 g of fruit plant was shed-dried, powdered and added to 1000 mL of 70% ethanol (v/v) and were left to macerate at room temperature for 20 h. The basin was slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 30°C.

**Acute toxic dose:** The intraperitonealy acute toxicity ($LD_{50}$) of the extract was evaluated in Swiss albino mice (Miller and Tainter, 1944). The method involved the administration of 5 different doses of the extract to 5 groups of mice (5 mice/group). After 24 h, there were no deaths in animals that received the plant extract at dose of 1, 10, 100 and 500 mg $kg^{-1}$ and just 1 death report at dose of 1000 mg $kg^{-1}$.

**Animals:** This study was approved by the Animals Ethics Committee from the Jerash National University, Jerash, Jordan (JNU).

Adult female Sprague-Dawley rats (40) weighing 250-300 g were used in this study. Rats were raised in the animal house unit (JNU) under a controlled temperature of 21±1.0°C and 12 h light/dark cycle. Animals were feed with regular diet (manufactured by the Faculty of Agricultural at (JNU), according to standard recipes) and water was provided *ad libitum*.

**Treatment with *Ballota undulata*:** Female rats were randomly divided into two treatment and control groups and the treatment group further subdivided into two subgroups; short and long duration groups.

*Ballota undulata* was dissolved in normal saline and the experimental rats receive treatment through an intra-gastric tube administration at a concentration of 300 mg $kg^{-1}$ b.wt. as one morning dose.

Experimental rats were divided into two groups: Group 1 consists of 10 female rats treated for a period of 4 weeks. Group 2 consist of 10 female rats treated for a period of 12 weeks. Group 3 and 4 consist of 20 female rats receive no treatment. All rats were allowed normal diet and access to drinking water for the same time periods.

**Fertility test:** Routine daily observation of rats exposed to *Ballota undulata* for clinical signs of toxicity was done. In addition, treated rats body weights were measured weekly.

After each treatment time period, treated and control groups of rats were divided randomly into subgroups of two female rats that were caged with a sexually mature male rat for ten days to allow mating. The effect of *Ballota undulata* ingestion on the occurrence of implantation was estimated in treated and their control counterpart's female rats after the appropriate time of mating exposure. It was estimated that at least two estrous cycles have elapsed during this exposure time (Lane-Petter and Pearson, 1971).

After the estimated mating time, treated and control counterparts female rats were weighted and sacrificed by cervical dislocation under light ether anesthesia. Autopsy was performed and the following parameters in both groups were recorded: the number of implantation sites, the number of viable fetuses and the number of resorption sites. Furthermore, uterus weights, ovary weight in addition to the embryo weights were recorded. Finally appropriate specimens of the uterus and ovaries were collected for histological studies.

**Histological studies:** The Bouin's fixed the excised of ovaries of females ingested the *Ballota undulata* drug, beside to their control were cut into small pieces and processed according to standard Eosin-Hematoxylin techniques. The paraffin embedding was followed by sectioning (5 μm) and staining (Harris haematoxyline and eosin). Finally slides were studied under light microscopy and results concluded.

**Statistical analysis:** Data was expressed as mean ± and Standard Deviation (SD). The differences between *Ballota undulata* treated and controlled groups were analyzed using Student t-test (Dixon and Massey, 1957).

## RESULTS

**Exposure toxicity of *Ballota undulata*:** None of the female rats used within the 4 week exposure group (group 1) showed any clinical signs of toxicity. However, one female rat exposed for 12 week treatment period with *Ballota undulata* (group 2) died.

Table 1a: The effect of 4 week exposure to *Ballota undulate* on fertility of female rats

| Treatments | No. of pregnant females | No. of implantation | No. of viable fetuses | Rats of resorption sites | No. of resorption sites/Total No. of implantation sites |
|---|---|---|---|---|---|
| Control | 9/10 | 9.33±2.39 | 8.87±2.72 | 4/10 (40.0%) | 5/84 (5.90%) |
| *Ballota undulate* | 8/10 | 8.65±3.54 | 7.90±0.50 | 5/7 (71.4%) | 17/70 (44.28%) |

Results are expressed as means±SEM, *p<0.05: Significantly different from the control group (Student's t-test), †p<0.05: Significantly different from the control group (Fisher exact test)

Table 1b: The effect of 12 week exposure to *Ballota undulate* on fertility of female rats

| Treatments | No. of pregnant females | No. of implantation | No. of viable fetuses | Rats of resorption sites | No. of resorption sites/Total No. of implantation sites |
|---|---|---|---|---|---|
| Control | 9/10 | 9.33±2.39 | 8.77±2.72 | 4/10 (40%) | 5/84 (5.9%) |
| *Ballota undulate* | 5/8† | 6.91±2.65* | 7.07±1.33* | 4/5 (80%) | 8/34 (23.5%) |

Results are expressed as means±SEM,*p<0.05: Significantly different from the control group (Student's t-test), †p<0.05: Significantly different from the control group (Fisher exact test)

Table 2a: The effect of 4 weeks exposure to *Ballota undulate* on maternal body, organ and embryo weights

| Treatments | Final body weight (g) | Ovary weight (g) (mg/100 g b.wt.) | Uterns weight (g) (mg/100 g b.wt.) | Embryo weight (g) (mg/100g b.wt.) |
|---|---|---|---|---|
| Control | 256±18.67 | 0.34±0.05 | 0.51±0.01 | 0.31±0.04 |
| *Ballota undulate* | 247±14.33 | 0.30±0.07* | 0.47±0.06 | 0.28±0.06† |

Results are expressed as means±SEM, *p<0.05, †p<0.01: Significantly different from the control group (Student's t-test)

Table 2b: The effect of 12 weeks exposure to *Ballota undulate* on maternal body, organ and embryo weights

| Treatments | Final body weight (g) | Ovary weight (g) (mg/100 g b.wt.) | Uterns weight (g) (mg/100 g b.wt.) | Embryo weight (g) (mg/100 g b.wt.) |
|---|---|---|---|---|
| Control | 256±18.67 | 0.32±0.05 | 0.49±0.01 | 0.31±0.04 |
| *Ballota undulate* | 234±15.65 | 0.25±0.03* | 0.43±0.08 | 0.26±0.32† |

Results are expressed as means±SEM, *p<0.05, †p<0.01: Significantly different from the control group (Student's t-test)

**The effects of *Ballota undulata* on fertility:** Short term treatment with *Ballota undulata* extract for 4 weeks revealed a slight decrease with no significant reduction in the rate of impregnation, the number of implantation sites, as well as the number of viable fetuses when compared with controls (Table 1a). A slight but not significant elevation in the percentage rate of resorption site was observed in this group when compared with controls. Furthermore, the ratio between the resorption and the total number of implantation was observed to be slightly elevated (Table 1a).

The effects of 12 weeks exposure to *Ballota undulata* by female rats (group 2) on the fertility indicate that there is a significant decreases in the percentage of impregnated rats in the treatment group when compared with the control counterparts (Table 1b). Moreover, Table 1b also indicates that the long term exposure to *Ballota undulata* for 12 weeks induces a decrease in both the number of implantation sites as well as the number of viable fetuses to a statistically significant level. It is also observed that the percentage of resorption sites in treated female rats for long term period is elevated, where the ratio between the resorption sites and the number of implantation was significantly affected (Table 1b).

**The effects of *Ballota undulata* on maternal and embryo organs weight:** Table 2a shows that ingestion of *Ballota undulata* for 4 weeks resulted in a slight but insignificant reduction in female rat's total body as well as uterine weights. A significance decrease in the relative ovarian and embryo weights in this group was observed when compared with control counterparts (Table 2a).

On the other hand, ingestion of *Ballota undulata* for 12 weeks resulted in a significant reduction in both the relative ovarian and embryo weights when compared to control group (Table 2b). No differences were observed in the final body weigh or in the uterine weight in rats treated for 12 weeks with *Ballota undulata* when compared with controls but to the contrary a slight reduction can be noticed (Table 2b).

**Histological effects of treated females rats:** All ovarian sections of females treated with *Ballota undulata* showed certain structural variation comparable to their control counterparts. The ovarian sections of females treated with 300 mg $kg^{-1}$ of *Ballota undulata* for 12 weeks shows beginning of corpus luteum degeneration due to active angiogenetic changes presented by presence of large number of congested blood vessels. Highly congested blood vessels were found surrounding corpus luteum and in the medulla. Some section showed decreased number of developing follicles, while in others normal developing follicles were absent. The number of atretic follicles ranges between low to high. Besides that, abnormal large fluid-filled cavities were present in most section. However, in the control group thicker corpus luteum besides significant number of developing follicles at primary, secondary and tertiary stages were present.

## DISCUSSION

The animal model in this study has been previously used by several other workers to assess the adverse effects of other extract obtained from medicinal plants on reproductive functions in rat males (Purohit and Daradka, 1999; Khouri and El-Akawi, 2005).

This study was conducted to investigate the exposure effect of *Ballota undulata* on the structure, fertility and the pregnancy outcome of adult female Sprague-Dawley rats. The dose of 300 mg $kg^{-1}$ b.wt. of *Ballota undulata* was selected to obtain broader range of information on the effects of this plant on the reproduction parameters. Two different time periods were selected namely 4 and 12 weeks.

It is worthwhile to mention that to our knowledge, no work has been published in the literature that relates the effects of *Ballota undulata* to structure, fertility and pregnancy outcome. It has been postulated however, that administration of this plant to female rats for 30 days in different dosages induces dose-dependent decrease in the size of the offspring with no toxicological effects observed (Shapira *et al*., 1989; Qazan *et al*., 2007).

This is in accordance with our results which showed that the exposure of adult female rats to *Ballota undulata* for 4 weeks had neither toxic, nor significant effects on the rat's fertility parameters or structure of the reproductive system. However, a slight decrease in the relative ovarian weights and a significant decrease in the embryo weight in rats treated for 4 weeks was observed. On the other hand, an increase in the exposure period for 12 weeks using similar dose of this plant extract revealed a significant decrease in both the relative ovarian and embryo weights when compared to controls.

Histological changes in ovaries of females treated with *Ballota undulata* extract provide a clear evidence of reproductive toxicity of *Ballota undulata* extract ingestion. Degeneration of corpus luteum impedes pregnancy maintenance in cases of successive implantation, leading to occurrence of early resorptions. Also, the arrestment of developing follicles at primary and secondary stages with the presence of decreased number of developing follicles at primary stage may result in reduction in the final number of mature graafian follicles produced. Congested blood vessels in corpus luteum and in medulla may interfere with material exchange and indicate an active resorption. Furthermore, the presence of degenerative areas and hypercellullarity in medulla may interfere with migration of developing follicles and impede their future development.

Other important findings of this study showed that this drug might promote a decreased in Sprague-Dawley female rats fertility when intra-gastric administration for long period of time was applied. This was indicated by the decrease in the reproductive organ weights observed in this group of rats. However, the weights of reproductive organs were markedly decreased as shown in Table 2a and 2b which might be explained by the fact that the reproductive organ weights can be closely regulated by androgen hormones (Richard *et al*., 2000). If so, we can hypotheses that this extract may act on the hypothalamic-pituitary ovarian axis which may lead to a decrease in the main hormones influencing oogenesis and subsequent pregnancy. The decrease in the weight of reproductive organs can be explained by the possible decrease in the level of androgen hormones that could be decreased in the experimental group of rats. The unexplained decrease in the ovarian weights in treated rats needs to be clarified through both hormonal and histological analysis. In addition, the future use of advanced molecular methodologies might elucidate the pathway through which this drug acts to decrease the weight of the ovaries observed in this study. These results, therefore, suggest that any disturbance of the reproductive endocrine functions may possibly and can go hand in hand with multiple sites of androgenic toxicity acting along the hypothalamic-pituitary-ovarian-uterine axis.

Other main finding of this current study was the significant reduction in the occurrence of pregnancy in rats exposed to *Ballota undulata* for 12 weeks. This decrease may be due to long dysfunctional period of the endocrine functions that might lead to decreased secretion of progesterone which is needed for endometrial alteration at the time of implantation and is necessary for successful impregnation (Choudhary and Steinberger, 1975; Agrawal *et al*., 1986; Qazan *et al*., 2007). This is in accordance with our results indicating the significant decrease in the number of implantation sites which could lead to the decrease in viable fetus's number. We are now conducting another study to investigate the effects of *Ballota undulata* exposure on serum progesterone levels.

In conclusion, the results of the current study suggest that ingestion of *Ballota undulata* by adult female rats causes adverse effects on fertility and reproduction.

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