Research Paper
The Effect of Aqueous Extract of *Origanum Vulgare* Linn on the Development of Genital Ridge in the Embryonic Stages of Rabbit

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**Abstract:** *Origanum vulgar* Linn, a light green color herb with strong aromatic odor and pleasantly bitter taste has widely been used in traditional medicine. We investigated the effect of this herb on the development of the genital ridge in embryo of the pregnant rabbits. For this study, we used three month old timed-mated New Zealand White (NZW) rabbits. They were fed with a standard diet and tap water ad libitum. *Origanum vulgar* was administered orally to female rabbits at 80 mg/kg/day orally, as a bolus while the control group received a volume of deionized water. Treatment for rabbits continued throughout mating until the F0 females reached gestation day 15. The histopathological assessment was performed on the genital ridge of either sex. Half were examined with light microscope while the other half in gluteraldehyde with electron microscope. Under light microscopy, the genital ridge has an epithelium cells and a thin layer of mesenchyme connective tissue, and two type of cells: the germ cells showed large rounded nuclei and pale-stained cytoplasm and the pre-granulosa cells were spindle shaped and smaller than the germ cells, having smaller nuclei and darkly stained cytoplasm similar as in control. Ultrastructurally, a primitive germ cell has an almost amoeboid appearance with eccentric spherical shape nucleus, and dense homogeneous nucleoplasm without evident aggregation of chromatin granules. The pre-granulosa cells are elongated, oval in shape with ellipsoid or spindle-shaped nuclei. The nucleus showed marginally condensed chromatin. The results indicated that the aqueous extract of *Origanum vulgare* does not have any toxic effect on the development of the genital ridge on embryo of pregnant rabbits.

**Keywords:** *Origanum vulgare* Linn, genital ridge, Germ and pregranulosa cells.
Introduction

*Origanum vulgare* Linn is a light green, aromatic, flavorful herb which has long been used in Spanish, Mexican and Mediterranean foods. It goes well with tomato sauces, pizza and vegetables (Gilani AH and Rahman Ail. 2005). Apart from culinary uses, it has also been used in traditional medicine for longtime. Researches provide evidences of its several medicinal properties. Oregano induces growth in growth retarded animals, and has nonspecific immunostimulatory effects on porcine immune cells (Williamson EM. 2001). This herb belongs to the family, \textit{Lamiaceae} and is distributed throughout Asia, Europe and North America (Fleming T. 1998). It is also known as Wild Marjoram and Winter Sweet in some countries such as Pakistan etc. (Baquar SR. 1989; Usmanghani K, et al 1997). In traditional medicine, oregano is widely used as lithotriptic, diuretic and antispasmodic. Other medicinal uses of the herb include stimulant, expectorant, antibacterial, anticancer, anti-inflammatory, antioxidant and laxative (Duke JA. 2002 and National Research Council, 1996).

*Origanum vulgare* Linn is rich in carvacrol (Bakkali et al., 2008) which used as a painkiller and it also known to reduce blood cholesterol and glucose level and suppress tumor activity (Goze et al., 2010). It can be used against respiratory and digestive disorders in menstrual defect (Gurudatt et al., 2010) as well. *Origanum vulgare* has antioxidant effects (Yoshino et al. 2006, Jalgoszyszyn et al. 2008) and protects against cell damage by oxidation (Lamaison et al. 1990). It contains some chemical substances which have an action similar to progesterone, and they provide normal growth (Zava et al. 1998, Arcila-Lozano 2004).

Certain drugs show toxic effect on the embryo and/or fetus in early pregnancy which leads to abortion (Scanley and Bower, 1986). Some medicinal plants have also showed similar toxic effect in mouse embryo (Lemonica et al. 1996; Benavides et al., 2001; Gutierrez-Pajares et al. 2003). The effect of aqueous extract of *Origanum vulgare* Linn on the early development of embryo in pregnant mice has already been established (Benavides et al., 2000). Therefore, our goal was to examine whether chronic exposure of *Origanum vulgare* Linn to rabbits could alter the development of the genital ridge of the embryo.

Materials and Methods

Animals and Husbandry

Timed-mated New Zealand White (NZW) rabbits were used, approximately 3 months of age at study initiation. The rabbits, weighed 2.3-3.4 kg, were procured from animal house of Faculty of Medicine Umm Alqura University. Rabbits were individually housed in stainless steel cages with wire-mesh flooring. Food (#5322 Purina Certified Rabbit Ration) was rationed at 65 g for the first 24 h for those females at gestational day (gd) 1, at 125 g for those females at gd 2 and available \textit{ad libitum} for all females from gd 3 to study termination. This gradual feeding to move the animals to \textit{ad libitum} was done to prevent the animals from overeating with possible development of mucoid enteropathy, as these animals were on food restriction during travel. Tap water was available \textit{ad libitum}. Animal rooms were maintained at 64-71°F and 44-64% humidity with a 12-h light/dark cycle. For mating, individual females were placed in the home cages of singly-housed males after given an intramuscular injection of 10 micro gram of gonadotropin-releasing hormone (GnRH) analog by 48 hours (animals were randomly mated on the basis of one male to one female). On the following morning and each morning thereafter, the females were examined for the presence of vaginal sperm or a vaginal copulation plug. The day on which the sperm or copulation plug was found was designated as gestational day (gd) zero. All animals were checked daily for clinical signs, mortality and evidence of abortion. Body weights and food consumption were measured at predetermined intervals throughout the course of this study. Following an acclimation period, they were individually housed (Van Rossum JM. 1963). Experiments were performed in compliance with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research
Council, and approved by the Ethical Committee for Research on Animals (ECRA) of Umm Alqura University, Saudi Arabia (Edeoga et al. 2005).

The plant of *Origanum vulgare* Linn was bought from Seekam Company, and identified by taxonomist. The aerial part of the plant material was cleaned of adulterants and kept soaked for three days in the aqueous-methanol (30:70) with occasional shaking at room temperature. The filtration was carried out using a muslin cloth and then through Whatman qualitative grade 1 filter paper. We repeated the procedure twice, then combined all the filtrates and finally concentrated on a rotary evaporator (RE-111, Buchi, Flawil, Switzerland) accompanied with B-700 recirculation chiller and a water bath model 461 at 40°C. A crude extract, yielding approximately 12% was obtained (Lemhadri et al., 2004 and Srihari et al 2008).

**Dosage**

Treated group was exposed to 80 mg /kg body weight of oregano via drinking water thorough Amber borosilicate glass water bottles fitted with a fluorocarbon septum containing stainless super tubes equipped with balls to minimize water dripping. Bottle systems were steam-cleaned twice weekly, and each water bottle was filled daily (Armitage, 1955). Water consumption during 24 h period was recorded. Dosing solutions were prepared based on average body weight and average daily water consumption (Cochran. 1954).

**Early Embryonic Development Study in Rabbits**

*Origanum vulgare* Linn was administered orally to female rabbits at 80 mg/kg/day orally, as a bolus. Control group animals in this study, received a volume of deionized water. There were 25 animals/sex/dosage groups. Animals were examined daily and clinical signs were recorded. Body weight and food consumption data were collected throughout the study for both control and treated females. It took 15 days after the mating period to produce the F1 generation, the offspring of F0 generation (parents). Treatment for rabbits continued throughout mating until the F0 (parental) females reached gestation day (gd) 15. F0 female were sacrificed via CO2 asphyxiation. The histopathological assessment was performed on the reproductive organs of either sex (left genital ridge). The specimens were divided into two half; one was fixed in 10% neutral buffered formalin, processed, embedded in paraffin wax, sectioned at 5 µm. They were stained with Hematoxylin and Eosin (H & E) for light microscopic examination and the other half in gluteraldehyde for electron microscope (Stuckhardt, J. L and Poppe, S.M. (1984). The crown length of the extracted embryos was measured to confirm their calculated age.

**Results**

**The Crown Length**

The crown length of the extracted embryos was 14-16 mm.

**At Prenatal Day –15**

In the control group, the genital ridge was poorly developed and it was formed with two types of cell (Germ and pre-granulosa) intermingled with each other. In the *Origanum vulgare* Linn treated group the result was similar to controls with few promotion germ cells of ameboid appearance were observed.

**Light Microscopy**

The genital ridge in the control negative was observed in close relationship with the mesonephros and the metanephros. It was covered by an epithelium consisted of more than one layer of flat cells. A
thin layer of mesenchymal connective tissue, the future tunica albuginea was observed beneath the covering of epithelium (Fig. 1). The bulk of the genital ridge in treated group was found to be packed with two types of cells without an evident organization. These cells were the germ cells which had large rounded nuclei and pale-stained cytoplasm, intermingled with the second type which termed as pre-granulosa cells. These pre-granulosa cells were small in size and spindle-shaped with smaller nuclei and darkly-stained cytoplasm compared with the germ cells (Fig. 2). Few capillaries were found within the ovary especially at the primitive medulla with PAS stain (Fig. 3).

**Electron Microscopy**

The cortex was packed with primordial ovarian follicles, consisting of a germ cell surrounding by almost a single layer of follicular cell, cluster. The ovarian parenchyma in control group was packed with two types of cells; the germ cells and the pregranulosa cells.

The germ cells, observed have rounded nuclei, occupying most of the cytoplasm with focally condensed chromatin and prominent nucleoli (Fig. 4). The chromatin granules tended to be aggregated particularly around the nuclear membrane and the cytoplasm of the germ cells contained round mitochondria, free ribosomes and vesicles of variable size (Fig. 5). However, in treated group, a primitive form of the germ cells was observed. This primitive germ cell had an almost ameboid appearance. Its nucleus was spherical in shape, eccentric in position and slightly indented with centrally located nucleolus (Fig. 5). The nucleoplasm was dense and homogeneous without evident aggregation of chromatin granules. The cytoplasm of the primitive germ cells contained mitochondria and well developed Golgi-complex (Fig. 6). The pregranulosa cells were elongated, oval in shape with ellipsoid or spindle-shaped nuclei. The nucleus showed marginally condensed chromatin (Fig. 8). The cytoplasm contained rounded mitochondria, endoplasmic reticulum, ribosomes and Golgi-complex.

**Discussion**

Some of medicinal plants have toxic effect during pregnancy leading to complications and effect on the embryo development (Scanley and Bower, 1986). It is important to know whether *Origanum vulgare* Linn has any effect on the reproductive development - either alert the development or provide normal development (Zava et al. 1998, Arcila-Lozano 2004). The known complication that may arise due to the lack of normal development of genital ridge is decrease the proliferation of germ cell (Chen et al., 2013) and effects numbers and direction of the germ cells (Golden et al., 1990), this alter the development of reproductive organs and gonadal differentiation. In the present study, using the light and electron microscopy to look any histological or ultrastructure of genital ridge cells, the genital ridge is the precursor to the gonads in close relationship with the mesonephros and the metanephros and formed mainly of mesenchyme and epithelial cells same as in human (Caouchard and Netter, 2002). In the genital ridge, we found that the germ cells were large rounded nuclei with pale-stained cytoplasm while the pre-granulosa cells were not only smaller than the germ cells but they were spindle shaped, having smaller nuclei and darkly stained cytoplasm. These observations were similar to the findings of Konishi et al. (1986) who reported similar data in human fetal genital ridge at prenatal ages. The ultrastructure of the germ cells of the prenatal rat and human genital ridge were already well-described in the literature (Eddy et al. 1974, Konishi et al. 1986, Pietta et al. 1998, 2000). According to the published reports, the germ cells have large rounded nuclei with dispersed nuclear chromatin and one or two prominent nucleoli. Their cytoplasm contained circular mitochondria as well as many free ribosomes. Our results are in agreement with these findings. We observed that the germ cells in genital ridge had large rounded nuclei with focally condensed chromatin and prominent nucleoli. Nuclear pores were also observed. The cytoplasm of the germ cells contained rounded mitochondria, free ribosomes, Golgi-complexes and infrequent fat droplets (Ocana-Fuentes et al., 2010; Sarac and Ugur, 2007). This indicates that aqueous extract of *Origanum vulgare* Linn does not have any toxic effect on the development, and it increases the rate of cell death similar to the findings of Domaracky et al., 2007 (in mouse preimplantational *in vivo*), and Benavides et al., 2010 (in
preimplantational mouse embryos). Therefore, our results which revealed that the *Origanum vulgare Linn* protects against cell damage (Lamaison et al., 1990) due to their antioxidant effects (Yoshino et al. 2006, Jalszyński et al. 2008), and provide normal development (Zava et al. 1998, Arcila-Lozano 2004) are in agreement with already published literature.

**Figures**

**Figure 1:** Photomicrographs of transverse section of the rabbit fetus (control), prenatal day-15, showing genital ridge in close relationship with the mesonephros (Ms) and the metanephros (Mt). The covering epithelium (E) formed of more than one layer of flat cells. Notice the mesenchymal connective tissue layer (arrowheads) beneath the covering epithelium. The genital ridges packed with the germ cells (short arrows) intermingled with the pregranulosa cells (long arrows). Notice the presence of small capillaries (C) in the primitive medulla (M) (Hx and E) (X300).

**Figure 2:** Higher magnification of the previous figure 1 showing the covering epithelium (E) formed of more than one layer of flat cells, and a mesenchymal tissue layer beneath it (arrowheads). The germ cells (short arrows) have large, rounded nuclei and pale cytoplasm, and the pregranulosa (long
arrows), have smaller spindle shaped nuclei and darker cytoplasm. Notice the metanephros (Mt; and the capillaries (C) (Hx and E) (X600).

**Figure 3:** Photomicrograph of transverse section of the rabbit fetus (treated with origanum), prenatal day-15, showing positive reaction with PAS stain of the coelomic epithelium (arrows) covering the mesonephros (Ms), and the ovary (O) (PAS) (X150).

**Figure 4:** Electron micrograph of rabbit ovary, prenatal day-15, showing the germ cells (G) having large round nuclei (N) with prominent nucleoli (n). Focal and marginal condensation of chromatin is seen. Notice the presence of a primitive germ cell (PGC) which has an amoeboid appearance. Spherical and eccentric nucleus (N) without chromatin aggregation (x10800).
**Figure 5:** Higher magnification of the previous figure showing the germ cells (G), having large round nucleus (N) with prominent nucleolus (n). The nuclear chromatin shows both focal and marginal condensation. Notice the well-developed nuclear pores (arrows), the primitive germ cell (PGC), the mitochondria (M) and ribosomes (r) (x16000).

**Figure 6:** Electron micrograph of rabbit genital ridge (*Origanum vulgare* group), prenatal day-15, showing both the germ cells (G) and the pre-granulosa cells (P). The germ cells nuclei (N) are rounded with focally condensed chromatin and prominent nucleoli (n). Notice the close opposition of the neighboring cells without any membrane specialization (X10800).
Figure 7: Electron micrograph of rabbit ovary (*Origanum vulgare* group), prenatal day-15, showing the germ cells, having the large nucleus (N) with a prominent nucleolus (n). The nuclear membrane shows well developed nuclear pores (small arrows). The cytoplasm contains rounded mitochondria (M). Golgi-complex (g), free ribosomes (r) and small translucent vesicles (v)(X26800).

Figure 8: Electron micrograph of rabbit ovary (*Origanum vulgare* group), prenatal day-15, showing the pre-granulosa cell (P), having an ellipsoid nucleus (N) with marginal chromatin condensation. Notice the close opposition of the neighboring cells without any membrane specialization. Notice the germ cell (G), the nucleus (N), nucleolus (n) mitochondria (M), Golgi-complex (g) and free ribosomes (r)(X16000).
Conclusions

Based on my results this study shows that the aqueous extract of *Origanum vulgare* Linn have no toxic affects histological and ultra structure development of genital ridge in the embryonic stages of rabbit.

References


