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Effects of aqueous extract of Origanum vulgare L. (Lamiaceae) on the preimplantational mouse embryos
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Introduction

Of the 250,000 species of flowering plants in the World, more than 20,000 – nearly 10% of the total – are classified as herbs. Herbs picked by people from the wild have been an essential factor in health care all over the World throughout the ages and in all cultures. Nowadays, some 80% of the World’s people rely on traditional, plant-based medicines for their primary health care (Cosge et al. 2009).

Actually, there exists a tendency to use natural products for the treatment of several illnesses. With use of medicinal plants, investigations have been performed all over the world in order to find more productive and economical medicines (Goze et al. 2010). Medications used to cure disorders require continuous changing to improve their effectiveness. However, few of the many claims of therapeutic efficacy have been validated adequately by clinical trials; Even though these claims have been substantiated scientifically, complementary medicines are unlikely to secure a place in conventional healthcare (Hammer et al. 1998).

The Labiatae family (Lamiaceae) is one of the largest and most distinctive families of flowering plants, with about 220 genera and almost 4000 species worldwide (Naghibi et al. 2005). Labiatae are best known for the essential oils common to many members of the family (Jones 1996, cited by Hammer et al. 1999). O. vulgare "oregano" is a herb widely used in cooking and natural medicine (Fon Quer 1985, Naghibi et al. 2005) that include many effective antioxidants, such as Rosmarinic acid, caffeic acid and various flavonoids (Yoshino et al. 2006).

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In a study on the phenolic acids recovered in human urine after single ingestion of Oreganum onites extract, Nurmi et al. (2006) identified phenolic constituent in the extract was rosmarinic acid, representing 75% of the identified phenolic acids. Other phenolic acids, including protocatechuic acid, p-coumaric acid, ferulic acid, chlorogenic acid and gallic acid, were present in the extract in notably lower amounts. The extract also contained minor amounts of the flavonoids luteolin and eriodictyol. In scientific reports about the medicinal properties of oregano: Oreganum oil, mainly rich in carvacrol (Bakkali et al. 2008), is used as a painkiller in rheumatism by rubbing externally on painful limbs. The aromatic oregano water, rich in carvacrol, is consumed to check gastrointestinal disorders, reduce blood cholesterol and glucose level and also for tumor suppressive activities (Goze et al. 2010); against whooping and convulsive coughs, digestive disorders and menstrual problems (Gurudatt et al. 2010), also its calming (Lans et al. 2007), antimicrobial (Nazia et al. 2007) and antifungal activity is emphasized (Ha & Glenn 2010).

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It is known that during the early pregnancy, the mammal embryo is susceptible to the toxic action of some drugs. These harmful effects depend on the exposure time and its concentration. At the undifferentiated stage of zygote, proliferation and before implantation, exposure to a teratogen usually either kills
the fertilized ovum which results in a spontaneous abortion, or spares it completely (Stanley & Bower 1986). During the recent years there have been reports on the toxic effects of some medicinal plants on the mouse embryo development (Lemonica et al. 1996, Gutierrez-Pajares et al. 2003). Although it is common to evaluate xenobiotic effects on mammalian embryos during organogenesis, the effects of these agents on preimplantation development is less commonly studied. Toxic agents or an unfavorable environment during preimplantation stages could alter normal events to arrest embryos or invoke abnormalities that affect subsequent stages of development. In a previous work, Benavides et al. (2000) found 10% of abnormal embryos when 20% aqueous extract of oregano was orally provided to pregnant mice in contrast to 14.29% of abnormal embryos found in the control group.

For this reason, it is necessary to evaluate and rule out the possible side or toxic effects that those plants could have (Benavides et al. 2001). The aim of this study was to examine the possible embryotoxicity of aqueous extract of O. vulgare on the mouse preimplantational embryo development.

Material and methods

Botanical samples were obtained from marketplace suppliers who had collected the plants from public lands. They were identified by Esther Cox, professor of Facultad de Ciencias Biológicas, Universidad Nacional Mayor de San Marcos. Voucher specimens of species studied are deposited in our laboratory.

Swiss Rockefeller strain mice from our animal house were maintained with a photoperiod of 14 h light/10 h dark, 22 – 24 °C room temperature and free access to balanced diet pellets (Purina, Peru). Females between six to eight weeks old were mated with proved fertile males between 8 – 12 weeks old. When a vaginal plug was observed, it was considered to be day one of pregnancy.

Aqueous extract.- Leaves of O. vulgare were dried at 60 °C in a stove for 24 h. The aqueous extract was prepared heating 300 g of oregano in 1500 mL of distilled water at 60 °C for 15 minutes, and then the extract was decanted. After filtration, a sample was separated for determination of the solid concentration (36 mg/mL). From this extract we prepared doses of O, 9, 18 and 36 mg/mL.

Experimental procedure.- Pregnant females were randomly assigned to four experimental groups: Group Control, Group A, Group B and Group C, each group (n= 10) was orally treated with 0, 9, 18 and 36 mg/mL ad libitum of aqueous extract of oregano, respectively since day one to day four. Female mice were euthanized by cervical dislocation 96 hours post copula (h.p.c.) and oviducts and uterine horns were excised, preimplantational embryos were collected by flushing oviducts and uterine horns with phosphate buffer saline (PBS, Sigma) pH 7.4 supplemented with 4 g/L bovine serum albumin (BSA. Sigma Chemical Co.) (Hogan et al. 1986).

Evaluation of embryo morphology and transport.- The stage and embryo quality were evaluated under a phase contrast microscopy and the grade of viability was determined by doing a morphological evaluation following Dorn & Kramer (1987) protocol with some of our own modifications (Figure 1):

Grade 1: there are no extruded blastomeres and the embryo has a rounded appearance.

Grade 2: there are extruded blastomeres and the embryo may not have a rounded appearance.

Grade 3: there are several extruded blastomeres and the embryo may be extremely malformed.

Degenerated: The embryo may be flat or bowl shape and most of the membranes are broken down.

Statistical analysis.- Data was evaluated by chi-square or Fisher test and all results with p<0,05 were considered statistically significant.

Results

This is not the first time that our laboratory demonstrated that oral administration of an aqueous extract of an herb causes damage to preimplantation mouse embryos (Gutierrez-Pajares 2003, Gonzales et al. 2007). In this time, this effect is not related to a systemic toxicity since there was no sign of intoxication in any of the doses in the pregnant mice.

Table 1 and 2 show the effects of treatment with aqueous extract of oregano on the early development stages of mouse embryo. Groups A and B did not show a significant difference.
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