Reproductive and developmental studies on Echinacea

In this guide

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Animal studies

31. There are no OECD guideline conforming in vivo studies on the reproductive and developmental toxicity of medicinally used Echinacea species. There are several studies investigating the effects of E. purpurea during pregnancy in mice (Barcz, E. et al., Chow et al., 2006) and pigs (Maass et al., 2005). The reproductive and immune parameters of E. pallida were investigated in pregnant rabbits (Dabbou et al., 2016) and their offspring (Kovitvadhi et al., 2016). No studies were found on the reproductive effects of E. angustifolia. The animal studies describing the reproductive and developmental effects of Echinacea are outlined below.

E. purpurea

32. Chow et al. (2006) investigated the potential association between E. purpurea consumption and spontaneous abortion in pregnant DBA/2 mice. Pregnant DBA/2 mice were randomised on the day a vaginal plug was detected to receive either E. purpurea–supplemented chow (n = 6) or standard Purina mouse chow (n = 7). An additional group of non pregnant mice (n = 7) received standard chow. The Echinacea supplemented diet was prepared by homogenising a commercially produced E. purpurea extract into finely ground standard chow, ensuring that individual mice consumed 0.45 mg/kg bw/day of the extract until they were killed (gestation days 10-14).

33. The study reported that Echinacea-fed mice showed reduced spleen lymphocytes and nucleated erythroid cells, aligning with levels in non-pregnant mice, whilst the bone marrow parameters were not influenced by the Echinacea supplementation. Although early pregnancy (days 10 – 11) showed no significant difference in foetal count, by days 12 – 14, only 50% of foetuses survived in the Echinacea group compared to controls (4.0/pregnancy in controls vs 2.0/pregnancy in Echinacea treatment group). The authors concluded that Echinacea may increase miscarriage risk in early pregnancy and advised against its use during this period.

34. Barcz et al. (2007) aimed to investigate whether pharmaceuticals containing dessicated alcoholic extracts of E. purpurea given to pregnant Balb/c mice influence the number, angiogenic activity and tissue vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in their foetuses. Eight female mice received 0.6 mg E. purpurea extract (see paragraph 35 for details) via an Eppendorf pipette from the 1st day of fertilization until the 18th day of pregnancy. Four mice were given vehicle control. On day 18, females were sacrificed and the foetuses were extracted, counted, weighed and homogenised for angiogenic assessment. Angiogenic activity was evaluated by injecting the

homogenate into two to three Balb/c mice and counting newly formed blood vessels on the inner skin surface. VEGF and bFGF concentrations in the homogenates were quantified using ELISA.

35. Within the experimental group, different E. purpurea formulations were used: three mice were given Esberitox, two received Immunal, and three received Echinapur. The authors did not report the compositions of these formulations. Based on an online search conducted by the Secretariat, Esberitox contains 3.2 mg dry extract (4–9:1) from a mixture of wild indigo rootstock, purple coneflower root, pale coneflower root, and arbor vitae tips and leaves (4.92:1; 85:1; and 85:1) per tablet; Immunal contains 1,140 mg of dry extract from fresh E. purpurea herb (1:12) and 60 mg of dry extract from fresh E. purpurea root (1:11) per tablet; and Echinapur contains 100 mg of thick extract from E. purpurea herb (DER 30–40:1; extractant: ethanol 23–30% v/v).

36. Barcz et al. (2007) reported that Echinapur and Esberitox groups showed a non-significant reduction in mean litter size compared to controls. However, all Echinacea treatments significantly reduced foetal VEGF and bFGF levels compared to controls ($p < 0.0001$). The angiogenic activity of the tissue homogenates, expressed as mean number of blood vessels, increased significantly in the Esberitox group, decreased in the Immunal group, and remained unchanged with Echinapur. The authors concluded that E. purpurea preparations may influence foetal angiogenesis and should not be recommended in pregnancy without further studies being carried out.

37. Maass et al. (2005) evaluated the effects of dietary E. purpurea in pregnant sows from day 85 of gestation to day 28 of lactation. Thirty-six sows were divided into three groups receiving 0%, 1.2%/0.5%, or 3.6%/1.5% Echinacea during pregnancy/lactation. The Echinacea supplement consisted of ground cobs made from the dried and pressed aerial parts of E. purpurea. Two batches were used, with constituent levels varying due to processing and storage: in the first batch, cichoric
acid declined from 420 to 290 mg/100 g plant dry matter and alkamides from 67.5 to 10.7 mg/100 g dry matter over the study period, while the second batch contained 170 mg/100 g dry matter cichoric acid and 44.1 mg/100 g dry matter alkamides. The study reported that Echinacea supplementation had no measurable effect on maternal liver enzyme activity (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase) or on haematological parameters, with no statistical differences observed between supplemented and control sows in leukocytes, erythrocytes, lymphocytes, granulocytes, neutrophils, eosinophils, basophils, or monocytes.

Colostrum crude protein content was 5% lower in the higher-dose Echinacea group compared with controls, although this difference was not statistically significant ($p = 0.11$). Maternal daily weight gain during gestation was 18% higher in the control group than supplemented groups, but weight loss during lactation did not differ between groups. Regarding offspring outcomes, piglet birth weight in the control group was 4% lower than in the Echinacea-supplemented groups, although this difference was not significant, and no significant differences were observed in the growth performance of suckling piglets.

E. pallida

38. Two linked studies investigated the effects of E. pallida supplementation in rabbits. In the first study (Dabbou et al., 2016), 100 pregnant does were fed either a standard diet or one supplemented with 3 g per kg of diet E. pallida from insemination to weaning. The Echinacea preparation contained caftaric acid, chicoric acid, chlorogenic acid and echinacoside with echinacoside found to be the main caffeic acid derivative. The study reported no differences between Echinacea supplemented and control does in maternal body weight at kindling, kindling rate, or blood morphology parameters, with red and white blood cell indices, platelet measures, and serum biochemical markers (total protein, glutamic oxaloacetic transaminase, blood urea nitrogen, albumin, urea, and cholesterol). Basophil counts were slightly reduced in the supplemented group, but this difference was not statistically significant. Regarding offspring outcomes, litter size at birth and at days 21 and 35, as well as kit mortality, did not differ between groups. Overall, the authors concluded that supplementation with E. pallida did not produce any significant effects on reproductive, haematological, or immune parameters in does.

39. The second study (Kovitvadhi et al., 2016) assessed the offspring of these does. Eighty weaned kits were allocated into four groups based on maternal diet and post-weaning diet: (1) offspring from control does fed the control diet, (2) offspring from control does fed the Echinacea supplemented diet, (3) offspring from Echinacea-supplemented does fed the control diet, and (4) offspring from Echinacea-supplemented does fed the supplemented diet. The diets consisted of a commercial basal feed with or without E. pallida supplementation (3 g per kg of diet). Parameters measured included growth, microbiome composition, blood biochemistry, phagocytic activity, and humoral immune response. The study reported that there were no significant differences in growth performances, blood parameters, bacterial community, or humoral immune response in the offspring.

Human studies

40. Human data on Echinacea use during pregnancy are limited and primarily based on observational studies and surveys. Two prospective studies (Gallo et al., 2000; Heitmann et al., 2016) reported no increased risk of major malformations, adverse pregnancy outcomes, or effects on birth weight or gestational age among women who used Echinacea, although information on species, preparation, dose, and duration was often incomplete. Survey data (Cuzzolin et al., 2010; Nordeng et al., 2011) also found no association between Echinacea use during pregnancy with adverse maternal or infant outcomes. Overall, the available human studies do not indicate adverse effects associated with use of Echinacea during pregnancy, but are limited by small sample sizes, reliance on self-reported use, lack of exposure characterisation, and potential confounding factors.

41. A prospective controlled study by Gallo et al. (2000) involving 206 pregnant women, enrolled and prospectively followed up after contacting the Motherisk Program, assessed the safety of Echinacea use during pregnancy. The study group was matched to a control group by disease (upper respiratory tract ailments), maternal age (±2 years), alcohol use, and cigarette use. The control group consisted of pregnant women who had contacted the Motherisk Programme regarding the safety of Echinacea for an upper respiratory tract ailment but subsequently did not use it or used a nonteratogenic antibiotic instead. In the study group, 112 women (54%) used Echinacea in the first trimester, with 17 (8%) exposed in all 3 trimesters. A total of 114 (58%) of 198 respondents used capsule or tablet preparations, or both, of Echinacea (250 to 1000 mg/d); 76 (38%) of the subjects used tinctures (5 to 30 drops per day). The self-reported duration of use was between 5 and 7 days. Different brands of E. purpurea and E. angustifolia were used, but the number of women using each species was not specified; E. pallida was only used by one woman. The study reported no significant differences between Echinacea users and controls in terms of pregnancy outcomes, including birth weight, gestational age, or malformation rates. Among Echinacea users, there were 195 live births, 13 spontaneous abortions, and 1 therapeutic abortion; the control group had similar outcomes (198 live births, 7 spontaneous abortions, and 1 therapeutic abortion). The authors stated that the malformation rates between Echinacea users and controls were also comparable, leading them to conclude that Echinacea use during organogenesis did not increase the risk of major malformations.

42. Heitmann et al. (2016) conducted a large prospective cohort study within the Norwegian Mother and Child Cohort, including 68,522 pregnancies after

exclusion of multiple births and chromosomal abnormalities. Maternal characteristics and potential confounders such as age, pre pregnancy BMI, folic acid use, smoking, education, previous pregnancy loss and year of delivery were adjusted for in the analyses using generalised estimating equations (GEE) models. Among the participants, 363 women (0.5%) reported using Echinacea during pregnancy, most commonly for treatment of respiratory tract infections. Echinacea supplements were taken during early (206 women) and late (183 women) pregnancy, though timing details were incomplete, and dosage/preparation were unspecified. The study reported that no increased risk of adverse maternal or pregnancy outcomes was observed in Echinacea users, and users did not show higher rates of preterm birth, low birth weight, or small for gestational age infants. Similarly, no increased risk of malformations was detected amongst the women who had used Echinacea during early pregnancy compared to controls; adjusted OR (95% CI) = 1.1 (0.6–2.1). There was 1.5% prevalence of major malformations in the women who had used Echinacea compared with 2.6% in the non-exposed group; adjusted OR (95% CI) = 0.6 (0.2–1.8). The three cases of major malformations that were detected among the Echinacea users were hypospadias, cleft lip, and hypoplastic left heart syndrome.

43. Cuzzolin et al. (2010) conducted a 10-month survey of 392 Italian women in maternity wards, using structured, face-to-face interviews to collect information on herbal product use during pregnancy, along with maternal health history and newborn outcomes. A total of 109 women (27.8%) reported using at least one herbal remedy during pregnancy, with 37.8% of them using herbal products throughout the entire gestational period. Echinacea was used orally by 10 women (9.2% of herbal users) for colds, anxiety, and immune support, although no details on species, plant part, preparation type, dosage, timing or duration were provided. By examining each herb descriptively, the authors reported one case in which prolonged Echinacea intake was possibly associated with intrauterine growth restriction in a 35 week newborn, although no further clinical details were provided for that case. The authors noted that the sample size limited the statistical analysis, resulting in all herbal products being treated as a single exposure group without distinguishing individual herbs and their separate effects on the pregnancy outcomes.

44. Nordeng et al. (2011) administered a structured questionnaire to 600 women within five days after delivery at Stavanger University Hospital Norway in order to investigate the use of herbal medicines in pregnant women in relation to pregnancy outcomes. In this cohort, 40% of women reported to have used herbal medicines during pregnancy, with Echinacea being used by 45 (7.5%) of those interviewed for cold and flu symptoms. No details were provided on Echinacea

species, dosage, or timing. In their analysis, the authors evaluated potential confounding factors through multivariable linear and logistic regression models, adjusting for maternal age, parity, education, marital status, gestational length, and conventional drug use, although some important confounders such as smoking and pre pregnancy BMI were not available. In this study, Echinacea use during pregnancy was not associated with adverse effects on birthweight, gestational age, mode of delivery, or neonatal complications.

Lactation

45. A case study examined the bioavailability of Echinacea alkylamides in human breast milk in a 35 year old volunteer at six different time points after ingestion of four Echinacea Premium tablets (Matthias et al., 2008). The tablets were prepared from dried ethanolic extracts of two Echinacea species and each tablet contained the equivalent of 675 mg E. purpurea root and 600 mg E. angustifolia root. A total of 13.1 mg of N-isobutyldodeca-2E,4E,8Z,10E/Z-tetraenamide alkylamides were ingested by the volunteer and they were found in the breast milk between 1 and 4 hours after the administration of the Echinacea tablets. Further details were not present in this conference abstract.

Maternal life history stages covered by reproductive and developmental studies

46. In order to identify any data gaps within the reproductive and developmental window when considering the safety of Echinacea in the maternal diet, the maternal life history stages covered by the animal and human studies discussed above are summarised in Table 1. Further information on the maternal life history stages, including their definition, can be found in the Scope of the Nutrition and maternal health project Annex. The terms insufficient, limited and adequate are used to describe the degree to which the study addresses and provides relevant data for the specific maternal life history stage. It must be noted that none of the studies are compliant with OECD guidelines.

Table 1: Maternal life history stages covered by available Echinacea animal and human studies

Study reference	**Study type**	***Echinacea* preparation and dose**	**Stage A (pre-mating to conception)**	**Stage B (conception to implantation)**	**Stage C (implantation to parturition - pregnancy)**	**Stage D (post-partum includi lactatio**
Chow *et al*. 2006	Animal study (DBA/2 mice)	*E. purpurea* extract 0.45 mg/kg bw/day (dose per body weight)	Insufficient	Limited	Limited	Insuffici
Barcz *et al*. 2007	Animal study (Balb/c mice)	*E. purpurea* extract 0.6 mg/day	Insufficient	Limited	Limited	Insuffici
Maass et al., 2005)	Animal study (pigs)	*E. purpurea* dried cobs 0.5-3.6%	Insufficient	Insufficient	Limited	Adequat
Dabbou *et al*., 2016	Animal study (rabbits)	*E. pallida* 3 g/kg	Insufficient	Adequate	Adequate	Adequat
Kovitvadhi *et al*., 2016	Animal study (rabbits)	*E. pallida* 3 g/kg	Insufficient	Insufficient	Insufficient	Adequat
Gallo *et al*., 2000	Human prospective controlled study	*E. purpurea* and *E. angustifolia* 250- 1000 mg/day	Insufficient	Insufficient	Limited	Insuffici

Heitmann *et al*., 2016	Human prospective cohort study	Not specified	Insufficient	Insufficient	Limited	Insuffici
Cuzzolin *et al*., 2010	Human cross-sectional study	Not specified	Insufficient	Insufficient	Limited	Insuffici
Nordeng *et al*., 2011	Human cross-sectional study	Not specified	Insufficient	Insufficient	Limited	Insuffici
Matthias *et al*., 2008	Human case report	Four tablets each containing *E. purpurea* 675 mg and *E. angustifolia* 600 mg	Insufficient	Insufficient	Insufficient	Limited