Toxicity Studies

In this guide

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In vitro and Animal Studies

Acute toxicity

77. Expressed juice from E. purpurea was administered either orally or intravenously to 8 week old Wistar rats and NMRI mice following the OECD guidelines for Good Laboratory Practice (GLP) and the OECD recommendations for technical methods at the time of the study (Mengs et al., 1991). Eight animals of each sex were given a single oral dose via gastric tube 15,000 mg/kg bw in rats and 30,000 mg/kg bw in mice. The intravenous dose was administered to eight animals of each sex via the tail vein at 5,000 mg/kg bw in rats and 10,000 mg/kg bw in mice. The animals were observed for 14 days and inspected several times daily and at the end of the experiment a necropsy with macroscopic inspection was performed. There were no deaths or any signs of abnormalities or toxicity due to the Echinacea. The authors concluded that a lethal dose cannot be found and LD50 was not calculated. Another study derived an estimated LD50 value 2,500 mg/kg bw from administering a mixture of polysaccharides from E. purpurea herb to 18 mice via intraperitoneal injection (Lenk 1989).

78. Acute toxicity study was performed with E. angustifolia following the OECD-423 criteria (Espinosa-Paredes et al., 2021). E. angustifolia was macerated, extracted with ethyl acetate and the extract was concentrated at low pressure in a rotary evaporator. The preparation contained 11.2 µg/mg echinacoside and 8.18 µg/mg caffeic acid. Three CD-1 male mice were administered 2,000 mg/kg bw of the extract and observed for 14 days to detect changes in behaviour and/or death. No signs of toxicity such as piloerection, mucosal irritation, motor activity alteration or death were recorded after the administration of Echinacea. There were no macroscopic morphological lesions to the lungs, kidneys, heart, stomach, intestines, spleen and liver of the animals. The authors classified the LD50 of the ethyl acetate E. angustifolia extract as Category 5 of the Globally Harmonized Classification System GHS (> 2,000–5,000 mg/kg), which means that it has a very low toxicity and could pose danger only to vulnerable populations.

Subacute toxicity

79. Expressed juice from E. purpurea was administered to groups of 18 Wistar rats per dose per sex at doses of 800, 2,400 or 8,000 mg/kg bw daily for 4 weeks via oral gavage (Mengs et al., 1991). During the study, food consumption was measured, and the rats were weighed once weekly. Biochemical and haematological parameters were determined at the start of the experiment and at weeks 2 and 4. At the end of the study the rats were submitted to an ophthalmological examination followed by necropsy. The organs were weighed, fixed in formalin and the histology was examined.

80. There was a statistically significant fall in plasma alkaline phosphatase in male rats at Echinacea doses 2,400 mg and 8,000 mg/kg compared to controls. A significant increase in prothrombin time was observed in female rats at doses of 2,400 and 8,000 mg/kg compared to controls. The authors concluded that since the alkaline phosphatase and prothrombin time were still in the normal physiological variation range for the rat strain used and there was no dose dependent response, no toxicological point of departure could be derived from the data. The other biochemical and haematological results, body weights, food consumption, ophthalmoscopy, necropsy findings and histology did not show any significant differences between the rat groups receiving the different Echinacea doses.

81. Espinosa-Paredes et al. (2021) conducted a 28-day repeated-dose toxicity study with the ethyl acetate extract of E. angustifolia described in paragraph 78. The extract was administered to five CD-1 mice per dose per sex at 20 mg/kg bw or 200 mg/kg bw. Serum asparate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine levels were determined. Authors reported that there were no statistically significant differences suggesting liver or kidney damage due to the extract administration.

Sub-chronic toxicity

82. The toxicity of E. purpurea extract was evaluated in a 13-week repeated oral dose toxicity test in Sprague Dawley rats (Jeong et al., 2024). The experiments were performed in accordance with GLP Regulation and Test Guidelines of Standards for Toxicity Studies of Drugs issued by the Korean Food and Drug Administration. The protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of BiotoxTech. The dried aerial parts of E. purpurea were powdered and extracted with 60% ethanol, followed by filtration and concentration. The concentrated extract was spray-dried for 1 day and standardised to at least 2% chicoric acid content. The extract was administered at daily doses of 500, 1,000 and 2,000 mg/kg bw to 10 rats per dose for each sex for 13 weeks. Food and water intake, body weight and clinical signs were monitored. Rats were sacrificed at the end of the treatment period and samples were collected for serum biochemical, urinalysis, and histopathological evaluations.

83. During the 13-week repeated oral toxicity study no mortality or abnormal clinical signs were observed in either sex at any of the tested doses (Jeong et al., 2024). There were no significant differences observed in body weight changes and food intake between the treatment and control groups. The ophthalmological

examinations revealed no signs of toxicity due to E. purpurea. The absolute organ weights and the organ-to-body-weight ratios at necropsy were not significantly different between the treatment and control groups for both sexes. The organ morphology was unremarkable. There were no significant differences in the haematology and serum biochemistry between the treatment and control groups for either sex. The urinalysis revealed some differences as the mean urine volume in the 1,000 mg/kg male rat group was significantly higher than in the controls. Some individual differences were also observed in the urinalysis, but they were not significantly different when compared to the controls.

Cytotoxicity

84. The cytotoxic effects of Echinacea flower extract and chicoric acid, bioactive constituent from Echinacea, were evaluated using human colorectal cancer cell lines HCT-116 and Caco-2 (Tsai et al., 2012b). The flowers of E. purpurea (L.) Moench were harvested from 6-month-old plants, freeze-dried, ground in a mill and sieved through 0.5 mm sieve. 10g of the powder was extracted with 50% ethanol, centrifuged, filtered, re-extracted with two more portions of 100 mL solvent. The solvent was then evaporated and freeze-dried with a vacuum to produce dry extracts. A previous study by the same authors showed that chicoric acid was the most abundant compound when E. purpurea was extracted with 50% ethanol (Tsai et al., 2012a). The other components of the preparation included caftaric acid, chlorogenic acid, echinacoside (Tsai et al., 2012a).

85. For the cell viability tests, the cell lines were treated with 0-2,000 µg/mL Echinacea extract and 0-200 µg/mL chicoric acid for 24-48 hours. The medium was removed, the cells were incubated for 2 h at 37°C with 5mg/mL MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The resulting formazan was solubilised with isopropanol, the absorbance was measured at 570 nm and the percent viability was calculated. The DNA fragmentation and telomerase activity of the cells were also determined. (Tsai et al., 2012b)

86. No significant difference in Caco-2 and HCT-116 cell viability was observed when the cells were treated with the different concentrations (0–2,000 µg/mL) of Echinacea extract for 24 hours. At 48 hours, the cell viability of both cell lines reduced in a dose-dependent manner. At 24 hours the chicoric acid showed a significant reduction in cell viability when the concentrations tested increased to 150 and 200 µg/mL, whilst at 48 hours all concentrations tested (50-200 µg/mL) showed a significant reduction in cell viability. Treatment of HCT-116 with 50-150 µg/mL chicoric acid reduced the telomarse activity of the cells determined by

telomeric repeat amplification assay. DNA fragmentation was also induced by chicoric acid in a concentration and time-dependent manner. Chicoric acid also led to caspase-9 activation and induced PARP cleavage at higher doses (100-150 µg/mL). The authors concluded that the possible in vitro cytotoxicity mechanism of E. purpurea extract is mediated by repression of telomerase activity, activation of caspase pathway and induction of apoptosis.

87. The cytotoxicity of ethyl acetate extract of E. angustifolia described in paragraph 77 was evaluated against two cancer cell lines (MDA-MB-231 ATCC HTB-26 and MCF-7 ATCC HTB-22) and a healthy breast epithelial cell line (MCF-10 ATCC) using an MTT cell viability assay (Espinosa-Paredes et al., 2021). The three cell lines (8 × 103 cells per well) were exposed to logarithmic concentrations (range 0.3-300 µg/mL) of the Echinacea extract for 24 or 48 hours. The absorbance was measured at 570 nm and the viable cell percentage was calculated. The assay was performed in triplicate in three independent experiments and the 50% inhibitory concentration (IC50) was calculated using GraphPad software. The E. angustifolia was not cytotoxic towards the healthy breast epithelial cell line (MCF-10 ATCC). The calculated IC50 of the extract towards the two cancer cell lines was between 16.3-28.8 µg/mL with no significant differences between the values obtained at 24 vs 48 hours.

Genotoxicity and Carcinogenicity

88. No genotoxic effects were observed in an in vitro bacterial reverse mutation assay, a mouse lymphoma assay, human lymphocyte assay and a micronucleus test performed by Mengs et al. (1991) using lyophilised E. purpurea expressed juice from the commercial product Echinacin Liquidum. The GLP OECD guidelines and the OECD recommendations for technical methods at the time of the study were followed. The details of the tests are described below.

89. E. purpurea was tested at concentrations from 8 to 5,000 µg/plate in an in vitro bacterial reverse mutation test in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538 with and without S9 metabolic activation (Mengs et al., 1991). The lyophilised Echinacea expressed juice, the bacteria and S9 mix were added to molten agar, mixed and poured onto minimal agar medium. 2-nitrofluorene 50 µg/plate, sodium azide 2 µg/plate, 9-aminoacridine 50 µg/plate and 2-aminoanthracene 5 µg/plate were used as positive controls, whilst dimethyl sulfoxide (DMSO) was used as the negative control. Plates were incubated at 37°C for 3 days and colonies were counted using an image analyser. The mean counts of 3 replicates were compared with the controls and the presence or absence of a

dose response was determined by linear regression analysis. The results were judged significant where reproducible, statistically significant and dose-related increases in revertant numbers were obtained. No significant increase in the number of revertants was observed, with and without metabolic activation, up to the maximum tested concentration of 5,000 µg/plate.

90. Lyophilised Echinacea expressed juice was tested for its ability to induce mutations at the hypoxanthine phosphoribosyltransferase (HPRT) locus of L5178Y mouse lymphoma cells, both in the presence and absence of S9 metabolic activation (Mengs et al., 1991). Lyophilised Echinacea expressed juice was used at concentrations of 50, 158, 500, 1580 and 5000 µg/mL. Two independent experiments were performed with triplicate cultures, each containing at least 1 x 107 cells. The cells were exposed to the test or control solution for 2 hours, washed and resuspended for a 7-day expression period. At the end of the expression period cells were plated in the absence and presence of the 6-thioguanine selective agent and the mutation frequency was determined after 14 days incubation. The induced mutations were considered significant if the lower 95th percentile of the mutant frequency of a treated culture exceeded the upper 95th percentile of the solvent control. Furthermore, the authors considered the overall test results as significant if the induced mutations occurred at consecutive doses in at least one of the experiments, and dose-related increases in mutation frequency could be confirmed by regression analysis. Cytotoxicity was measured by determination of plating efficiency relative to the control, immediately post-treatment with Echinacea. The Echinacea treatment at the tested doses did not result in a statistically significant increase in the mutation frequency, either in the presence or absence of S9 metabolic activation. The Echinacea doses up to the maximum tested (5,000 µg/mL) were also considered virtually non-toxic to the cell line.

91. The lyophilised Echinacin Liquidum was also tested for its ability to induce chromosomal aberrations in an in vitro cytogenetic assay using human lymphocyte cultures from a male donor. The cells were incubated with 2400, 3500 or 5000 µg/mL Echinacea for 20 or 44 hours (in the absence of S9) or 3 hours followed by a 17- or 41-hours recovery period (in the presence of S9). Prior to harvesting the cells, colchicine was added to arrest them in metaphase 1.5 hours before harvest. The cells were fixed and examined microscopically for mitotic index. One hundred metaphases from each treatment were analysed for chromosomal aberrations. The results were considered significant where statistically significant increases in the proportion of structurally abnormal cells occurred at one or more concentrations and exceeded the normal range for this

laboratory (0-4 cells with structural aberrations excluding gaps per 100 cells). There was no evidence of mitotic inhibition following the Echinacea treatment of up to 5,000 µg/mL in both the presence and absence of S9 mix at either sampling time (20 or 44 hours). A small, but statistically significant increase in the proportion of cells with structural aberrations was observed at 5,000 µg/mL at the 20 h sampling point in the absence of S9. The authors commented that this was not considered to be of biological significance as the frequency of aberrant cells fell within the range of biological control and it also coincided with an increase in the osmolality of the treatment medium.

92. Mengs et al. (1991) also performed an in vivo micronucleus test using 25,000 mg/kg Echinacin Liquidum administered as a single dose via oral gavage to groups of 5 male and 5 female mice. The animals were sacrificed at either 24, 48 or 72 hours after the dose. The positive control animal groups were given intraperitoneal injection of 100 mg/kg cyclophosphamide, whilst the negative control groups were given 25 mL/kg water via oral gavage. Bone marrow was collected from the femur and two bone marrow smears were prepared from the cells of each animal by fixing and staining. One thousand polychromatic erythrocytes (PCE) were analysed microscopically for the presence of micronuclei. The positive control showed a significant increase in the proportion of micronucleated PCE, whilst the Echinacea preparation did not show any statistically significant differences compared to the negative control.

93. The mutagenicity and the antimutagenic effects of E. purpurea were tested in S. typhimurium TA 98 and TA 100 strains with and without S9 metabolic activation (Tsai et al., 2012a). The E. purpurea extracts were prepared as described in paragraph 82. For the toxicity test each plate was inoculated with 0-5 mg E. purpurea extract and the viability of the bacterial strain was evaluated after 48-hours incubation at 37°C. For the mutagenicity test, the bacteria were mixed with 0.25-5 mg/plate Echinacea extract, incubated on histidine containing media at 37°C for 48 h and the reversion rate was compared to the control plate. The antimutagenic activity of the Echinacea extract was evaluated by calculating the % inhibition of the reversion rate with and without E. purpurea in the presence of mutagenic compounds.

94. The freeze-dried extract of E. purpurea showed no toxicity against S. typhimurium strains TA98 and TA100 concentrations of ≤5.0 mg/plate, with or without S9 metabolic activation (Tsai et al., 2012a). None of the tested concentrations of E. purpurea showed any significant differences in the revertant number with or without S9 mix. The Echinacea extract showed a dose-dependent

inhibitory effect on the mutagenicity of 2-aminoanthracene in both S. typhimurium strains.

95. The E. purpurea extract described in paragraph 82 was tested for genotoxicity using an in vitro bacterial reverse mutation test in accordance with OECD Guideline No. 471, in vitro chromosomal aberration test in accordance with OECD Guideline No. 473 and an in vivo micronucleus test in accordance with the OECD guideline No. 474 (Jeong et al., 2024). The paper concluded that the tests did not reveal any evidence of genotoxicity as there was no significant increase in the number of mutant bacterial colonies even at the maximum concentration tested. Furthermore, the chromosomal aberration and micronucleus tests didn't show any significant differences in chromosomal damage compared to controls. Details of the tests are described below.

96. For the in vitro bacterial reverse mutation test the extract was diluted two-fold in water and the highest concentration tested was 5,000 µg/plate. S. typhimurium strains TA98, TA100, TA1535 and TA1537 and tryptophan auxotroph mutant Escherichia coli strain (WP2uvrA) were used. Sodium azide, 2-nitrofluorene, 2-aminoanthracene, aminoacridine and 4-nitroquinoline N-oxide were used as positive controls and water was used as negative control. The Echinacea extract was incubated with a 109 cells/mL suspension of each strain for 20 minutes at 37°C, with and without S9 mix, poured on agar plates to solidify and incubated for 48 hours prior to checking for revertant colonies. No growth inhibition was observed at any of the doses of E. purpurea tested for the bacterial strains. The authors reported that the number of mutant colonies in the E. purpurea groups at all doses tested, with and without S9 metabolic activation mix, did not exceed twice the negative controls, whilst the positive controls used yielded between 5 to 37 times more colonies than the negative controls. (Jeong et al., 2024).

97. The in vitro chromosome aberration test was performed using mammalian cultured cell lines Chinese Hamster Lung (CHL/IU) cells seeded at a density of 5 × 104 cells/mL in a 60 mm plate and treated with E. purpurea extract (78,1, 156 and 313 µg/mL) for either 6 or 24 hours. Mitomycin C, 0.1 µg/mL and benzo[a]pyrene, 20 µg/mL were used as positive controls, whilst water was used as negative control. Microscopic slides for chromosomal observation were prepared and chromosomal abnormalities were divided into structural and numerical aberrations. No statistically significant difference was observed in the frequency of cells with chromosomal aberrations, in the absence or presence of S9 metabolic activation, in both short-term 6 hour and the 24-hour continuous

treatments with E. purpurea extract compared to the negative control. With the positive controls there was significant increase ($p>0.01$) in the cells with structural abnormalities compared to negative controls. (Jeong et al., 2024).

98. The E. purpurea extract was administered to seven-week-old male Sprague Dawley rats at a two-fold dilution dose range from 1,250-5,000 mg/kg bw with 5 animals per dose for the in vivo micronucleus test. After the second dose, the animals were sacrificed, their femurs were dissected and perfused with phosphate buffer saline (PBS) to collect the bone marrow cells. The bone barrow cells were fixed with 10% formalin and stained with 0.05% acridine orange. Fluorescence microscope was used to count the numbers of micronucleated polychromatic erythrocytes (MNPCE) and polychromatic erythrocytes (PCE) in red blood cells (RBCs). The genotoxicity index was expressed as the average number of MNPCE among 4,000 PCE per rat, and the cytotoxicity index was expressed as the average number of PCE among 500 RBCs. The cytotoxicity index was similar in all groups tested. The genotoxicity index calculated was a 100-fold higher in the positive compared to the negative control, but was not statistically different between the different doses of Echinacea and the negative control. (Jeong et al., 2024).

99. The mutagenicity of E. angustifolia was tested using an in vitro bacterial reverse mutation test in S. typhimurium TA98, TA100 and TA102 in the presence and absence of S9 metabolic activation mix (Espinosa-Paredes et al., 2021). The ethyl acetate extract of E. angustifolia described in paragraph 78 was tested at concentrations between 50 and 200 µg per plate. The plates were incubated at 37°C for 48 hours and the number of mutant colonies were counted. Picrolonic acid (50 µg/plate), 2-amino-anthracene (10 µg/plate), N-methyl-N′-nitro-N-nitrosoguanidine (10 µg/plate) and mitomycin-C (10 ng/plate) were used as a positive control, whilst 0.1% dimethyl sulfoxide (DMSO) was used as negative control. A test was considered positive when the number of spontaneous colonies exceeded twice the number of basal revertants. The authors reported that the tested concentrations of E. angustifolia extract, with or without S9 mix, did not yield a positive test and no genotoxic activity was therefore observed. The positive controls on the either hand yielded between 7 to 55 times more colonies than the negative control.

100. Espinosa-Paredes et al. (2021) also performed an in vivo micronucleus test using male CD-1 mice. The ethyl acetate E. angustifolia extract was administered intragastrically at 1,000 mg/kg bw to 3 mice. 1% DMSO was used as a vehicle control, water as the negative control and cyclophosphamide (50 mg/kg) as the

positive control. The animals were euthanised 48 hours after test substance administration, blood samples were collected, fixed and labelled prior to flow cytometry analysis. The number of normochromatic erythrocytes (NCEs) and reticulocytes (RETs), with and without micronuclei (MNs), was determined in order to calculate the percentages of mature normochromatic erythrocytes (% MN-NCEs), micronucleated reticulocytes (% MN-RETs) and the percentage of total reticulocytes (% RETs). The E. angustifolia extract did not lead to a significant increase in the formation of micronuclei yielding a 0.9 % MN-RET compared to 0.3% in the negative control. There was a significant increase in the MN-RET (8.4%) in the positive control compared to the vehicle control (0.4%). There was a decrease in the frequency of RET in the Echinacea extract group compared with the negative control (2.56% vs 5.41%, $p < 0.05$), but the authors have not commented on that, and its significance is unclear.

101. An in vitro carcinogenicity study was performed using lyophilised E. purpurea extract using Syrian hamster embryo cells (SHE) (Mengs et al., 1991). Six different concentrations of Echinacea in the range 5-55 µg/mL were tested and benzo(a)pyrene at 1.25 and 2.5 µg/mL was used as a positive control. Two independent experiments were performed with 20 replicates per concentration tested. The cells were incubated with the Echinacea or control for 7 days, washed, fixed with methanol and stained with 10% Giemsa. A stereomicroscope was used to evaluate the cells for altered or transformed morphology and average of 40 colonies per dish were examined. There was no significant difference in the frequencies of morphologically transformed colonies between the Echinacea and the negative control. The authors concluded that there was no evidence that Echinacea induces malignant transformation in SHE.

Reproductive and Developmental Toxicity

102. There are no guidelines conforming in vivo studies on the reproductive and developmental toxicity of medicinally used Echinacea species. There is a study looking at the effects of several herbal medicines, including E. purpurea, on human sperm motility (Ondrizek et al., 1999). 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.

103. A study investigated the effects of E. purpurea on human sperm motility (Ondrizek et al., 1999). Fresh donor sperm were washed and incubated with either 0.81 mg/mL or 8.1 mg/mL E. purpurea for 48 hours at 37°C. The lower of the tested concentrations was based on one-thousandth of the recommended daily dose dissolved in 1 mL of medium. Sperm aliquots were analysed at 0, 1, 4, 24 and 48 h of incubation. Sperm dimensions and kinematic parameters were measured using the Hamilton Thorn motility analyser. There were no significant differences in the kinematic parameters and the sperm motility between the medium control and lower concentration (0.81mg/mL) of Echinacea. However, inhibition of sperm motility was observed at 48h with the higher E. purpurea concentration (8.1 mg/mL) tested.

104. The possible association between consumption of E. purpurea during pregnancy and spontaneous abortions in mice was investigated by Chow et al. (2006). The authors based their investigation on the implications of natural killer (NK) cells on foetus rejection, which manifests in spontaneous abortions (De Fougerolles and Baines, 1987; Gendron and Baines, 1988). Previous studies have shown that Echinacea significantly increases the number of NK cells in both healthy (Currier, 2000) and leukemic mice (Currier and Miller, 2002). Commercially prepared E. purpurea extract was homogenized into finely ground standard chow that individual mice consumed at 0.45 mg/day. Pregnant mice (n=6) were fed the Echinacea containing chow from pregnancy onset until gestational days 10-14 until females were killed. Half of the mice (n=3) were killed at day 10-11 gestational days (early pregnancy) and the other half (n=3) at 12-14 gestational days (mid pregnancy). Foetuses from 3 pregnancies in both gestational age groups (10–11 days and 12–14 days) were removed and preserved in fixative for subsequent counting. Maternal spleen and bone marrow were taken for enumeration of cells in each of five separate hemopoietic lineages/organ.

105. The study (Chow et al., 2006) reported that the number of spleen lymphocytes and nucleated erythroid cells, normally increased during pregnancy, were decreased in Echinacea-fed mice back to levels not significantly different from those of non-pregnant animals. The bone marrow parameters were not influenced by the Echinacea supplementation. The results also indicated that miscarriages are more likely to happen in the early stages of pregnancy (10–11 days) in the Echinacea-fed mice. At days 10-11, there are no significant differences in the average number of foetuses between Echinacea-fed mice and controls (4.7/pregnancy in controls vs 4.0/pregnancy in treatment group). Foetal loss (resorption) appears to begin early, prior to the first recording (days 10–11)

in E. purpurea-consuming pregnant mice, and only 50% of the foetuses from Echinacea-consuming pregnant mice survive by day 12–14 of gestation compared to controls (4.0/pregnancy in controls vs 2.0/pregnancy in treatment group). Based on these results, the study suggests that pregnant women should avoid the consumption of Echinacea in the early stages of pregnancy in women. It is worth bearing in mind that this study investigated only one E. purpurea dose in a small number of animals with supplementation starting after the pregnancy was established.

106. Barcz et al. (2007) investigated the effects of Echinacea on the angiogenic activity and tissue VEGF and bFGF in foetuses from pregnant mice exposed to E. purpurea extracts. Eight female mice were given 0.6 mg E. purpurea extract via an Eppendorf pipette from the 1st day of fertilization until the 18th day of pregnancy. Four mice were given vehicle control. In the experimental group, different Echinacea formulations were used with three mice fed Esberitox, two Immunal and the remaining three Echinapur. On day 18, the females were sacrificed, the foetuses were extracted, counted and weighed. Foetuses from each litter were pooled and homogenised for further angiogenic evaluation. The angiogenic activity of the tissue homogenate was performed by injecting the homogenate in two to three Balb/c mice and counting newly formed blood vessels on the inner skin surface. The concentrations of VEGF and bFGF in the homogenate were determined by ELISA.

107. The study (Barcz et al., 2007) reported that that two of the Echinacea preparations, Echinapur and Esberitox, slightly lowered the mean number of foetuses (n=7.6) per litter when compared to Immunal (n=9.5) and the control (n=10), but results were on the border of statistical significance ($0.05<p<0.01$). The mean VEGF and bFGF foetal tissue concentrations from mothers exposed to all Echinacea preparations were significantly decreased compared to controls ($p <0.0001$). The angiogenic activity of the tissue homogenates, expressed as mean number of blood vessels, was significantly increased in the Esberitox group (28.2 ± 6; $p<0.0001$), but decreased in the Immunal forte group (18.2 ± 5.4 $p < 0.01$) when compared to standard/control diet (22.5 ± 4.3). No significant difference in the angiogenic activity of the foetal tissue homogenate was observed with Echinapur (21.3 ± 4). The study concluded that E. purpurea preparations may influence foetal angiogenesis and should not be recommended in pregnancy without further studies being carried out (Barcz et al., 2007).

108. In another study (Maass et al., 2005), E. purpurea was introduced in the diet of pregnant sows in the form of dried cobs consisting of the aerial part of the

plant. The study was carried out on the total of 36 sows, divided into three groups of 12, from the 85th day of pregnancy to the 28th day of lactation. Echinacea was supplemented in the diet of the three groups during 85-110th day of pregnancy at a dose of 0%, 1.2%, or 3.6%. The Echinacea supplementation was continued until the 28th day of lactation at 0%, 0.5%, or 1.5%. During gestation, the feed amount (2.6 kg/sow/day) was set to match the nutritional requirements of the sows. The lactation diet was offered ad libitum with a maximum of 6 kg/sow/day. Blood analysis was performed on day 85 of pregnancy as well as on day 1 and 28 of lactation. The colostrum was collected manually from 1 to 6 hours after delivery. Body mass, body temperature, health status, crude protein, immunoglobulins, haematological, and clinical chemistry parameters (alkaline phosphatase, alanine, and aspartate, and gamma-glutamyl transferase aminotransferase) were analysed using the acquired samples.

109. No influence of Echinacea on the enzyme results (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase) was observed. There was no statistical difference in the levels of leukocytes, erythrocytes, lymphocytes, granulocytes, neutrophils, eosinophils, basophils, or monocytes between the Echinacea supplemented and the control groups. The level of crude protein in colostrum was 5% lower in the higher dose Echinacea supplemented group compared to the control, but this difference was not statistically significant ($p = 0.11$). Daily weight gain of the sows during gestation was 18% higher in the control group than in the Echinacea supplemented groups. During lactation no differences in the average weight loss were found. The piglet body weight at birth in the control group was 4% lower than in the supplemented groups. However, this difference was not significant and the growth performance of the sucking piglets also showed no significant differences between the control and supplemented groups. (Maass et al., 2005).

110. The effects of E. pallida dietary supplementation on the reproductive performance, blood parameters and immune indices were studied in pregnant rabbits (Dabbou et al., 2016). The Echinacea preparation contained caftaric acid, chicoric acid, chlorogenic acid and echinacoside with echinacoside found to be the main caffeic acid derivative. The does were randomly assigned to two groups (50 does/group). The first group was fed a commercial pelleted diet ad libitum, while the second one was fed the same diet supplemented with 3 g/kg E. pallida. Echinacea was given from insemination to kit weaning. The study measured the reproductive performance of the does based on the following parameters: total born; born alive; stillborn; litter size at 21 and 35 days of age; litter weight at 21 and 35 days of age; individual body weights of kits at 21 and 35 days of age;

kindling rate (%) = number of kindled does per number of inseminated does × 100; prolificacy = number of born kits per number of does kindled; numerical productivity at birth = number of born alive per inseminated doe; overall productivity at birth = weight of born alive per inseminated doe; perinatal mortality (%) = number of stillborn kits per number of total born × 100; mortality between 0 to 21 and 0 to 35 days of age. Full haematological parameters and blood biochemistry were performed on days 0, 14, and 28 of the experiment. Serum lysozyme was measured as part of the innate immunity evaluation.

111. The body weight of the does at kindling, the kindling rate and the litter size at birth and at days 21 and 35 of age, and the mortality of the kits did not differ between the two groups. The blood morphology parameters of mothers did not significantly differ between treatment and controls: red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean haemoglobin concentration, red blood cell distribution width, platelet count, mean thrombocyte volume, mean platelet volume, platelet distribution width, number of white blood cells, lymphocytes, monocytes, neutrophils and eosinophils. The Echinacea supplemented group showed reduction in basophil cell rate, but it was not significantly different from controls. The concentration of total protein, glutamic oxoloacetic transaminase, blood urea, nitrogen, albumin, urea and cholesterol were also analysed and were not different between treatment and control groups. The serum lysozyme assay didn’t yield significant differences either. Overall, the authors concluded that supplementation with E. pallida did not show any significant effects on the reproductive, haematological, or immune parameters of does.

112. The second part of the above study aimed to evaluate the effects on the performances, bacterial community, blood parameters and immunity of E. pallida dietary supplementation in the offspring of the control and Echinacea supplemented group (Kovitvadhi et al., 2016). Eighty kittens were taken from the control group (C) and Echinacea (E) groups of the previous study (Dabbou et al., 2016). The kittens were weaned at 35-days old, randomly separated into four groups of 20 and were housed in individual wire cages. The growing rabbits were fed a growing commercial basal diet with or without the supplementation (3 g/kg of E. pallida): the CC group (rabbits from the control does fed the control diet), CE group (rabbits from the control does fed the supplemented diet), EC (rabbits from the Echinacea does fed the control diet) and EE group (rabbits from the Echinacea does fed the supplemented diet). The hard faeces of the growing rabbits were collected at 35, 49 and 89 days from five animals per group for microbiome analysis. Blood samples were taken from 10 rabbits per group at 89 days of age

for haematological analysis. Renal and liver function and lipid metabolism were also measured. Phagocytic activity was determined in an assay against Streptococcus canis, which looked under immersion light microscope for neutrophils and monocytes that engulfed the Gram-positive cocci. Humoral immune response against vaccination was also studied by measuring antibody production in response to vaccination against rabbit haemorrhagic disease virus. The study reported an increase in phagocytosis in rabbits fed diets supplemented with E. pallida, regardless of the Echinacea supplementation status of maternal diet. There were no significant differences in growth performances, blood parameters, bacterial community, or humoral immune response.

113. A study investigated the ability of levamisole and ethanolic (70%) extracts of E. purpurea dried aerial parts to prevent teratogenic effects such as cleft palate induced by phenytoin (Khaksary Mahabady et al., 2006). Thirty two pregnant NMRI mice of age 6-8 weeks were divided into four groups (n=4) as follows: group 1 received normal saline (10 mL/kg), group 2 received phenytoin (65 mg/kg), group 3 received phenytoin (65 mg/kg) and 12 h later levamisole (10 mg/kg), whilst group 4 received phenytoin (65 mg/kg) and 12 h later extract of E. purpurea (360 mg/kg). All drugs were administered intraperitoneally from the first day of gestation, which was assumed to be upon the discovery of vaginal plug following mating. The mice were sacrificed on gestational day 19, the foetuses were collected (total number of collected foetuses from groups 1-4 were 64, 81, 77 and 61 respectively), stained by Alizarin red–Alcian blue method and investigated by stereomicroscope for cleft palate. In the saline control group the palatal closures of all foetuses were normal, whilst the incidence of phenytoin induced cleft palate was 16%. Levamisole reduced incidence of pheytoin-induced cleft palate to 5.3%, whilst the E. purpurea extract reduced it to 3.2%. Equally, the mean weight and length of the foetuses were significantly decreased in the phenytoin group ($p<0.001$), whilst the groups that received Echinacea and levamisole had similar parameters to the saline control. The authors concluded that the observed protective activity of levamisole and Echinacea against phenytoin-induced cleft palate was due to immunomodulating and anti-inflammatory effects of these agents.

Human Studies

Exposures in pregnancy

114. A systematic review conducted on the available literature up to 2006 (Perri et al., 2006) concluded that good scientific evidence from a prospective follow up

study (Gallo et al., 2000) showed that oral consumption of Echinacea during the first trimester does not increase the risk of major malformations. Low level evidence based on expert opinion from a botanical medicine panel concluded that Echinacea is not teratogenic and oral consumption of Echinacea in recommended doses is safe during pregnancy and lactation. However, the expert panel advised that caution should be exercised until there is stronger evidence on safety of Echinacea during lactation. (Perri et al., 2006).

115. The first prospective controlled study aiming to evaluate the safety of Echinacea use during pregnancy consisted of 206 women who were enrolled and prospectively followed up after contacting the Motherisk Program (in Toronto, Canada) regarding the gestational use of Echinacea (Gallo et al., 2000). There was a disease-matched control group of 206 women to control for maternal age, alcohol consumption and smoking. In this 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 predominantly used, but the number of women using each species is not specified. E. pallida was only used by one woman. No information was provided on the part of the plant used in these preparations.

116. No statistical difference was reported between the Echinacea users and disease-matched controls in terms of pregnancy outcome, delivery method, maternal weight gain, gestational age, birth weight, or foetal distress. There were 195 live births, including 3 sets of twins; 13 spontaneous abortions; and 1 therapeutic abortion in the treatment group. The disease-matched control group reported 198 live births, 7 spontaneous abortions, and 1 therapeutic abortion. (Gallo et al., 2000).

117. Rates of malformations between the treatment and control groups were also not statistically significantly different. There were 6 major malformations including 1 chromosomal abnormality, and 6 minor malformations in the Echinacea exposed group. With first-trimester use of the herb, 4 major and 2 minor malformations were reported. In the control group, 7 major and 7 minor malformations occurred. Overall, the authors concluded that gestational use of Echinacea during organogenesis is not associated with an increased risk for major malformations (Gallo et al., 2000).

118. The Norwegian Institute of Public Health conducted The Norwegian Mother and Child Cohort Study, a prospective population-based pregnancy cohort study (Heitmann et al., 2016). Pregnant women in Norway were recruited through a postal invitation in connection with a routine ultrasound examination offered to all pregnant women around pregnancy week 17. Women filled in 3 questionnaires at week 13-17, week 30 and when the child was 6 months old. Information on Echinacea use was retrieved from the questionnaires. Maternal age, pre-pregnancy BMI, folic acid use, smoking, education, previous miscarriages/stillbirths, the year of delivery were considered as possible confounders and adjusted for. In addition, low birth weight was adjusted for by length of gestation. Women who gave birth to multiples (n=1,291) and children with chromosomal malformations (n=121) were excluded from the study design. The study population consisted of 68,522 pregnancies; amongst these 68,198 (99.5 %) resulted in a live birth, 219 (0.3 %) resulted in a stillbirth, and 104 (0.2 %) resulted in a neonatal death. The mean birth weight and the median gestational age among live-born infants was 3,605 g and 40 weeks, respectively. Any malformation occurred in 3,201 (4.7 %) of the pregnancies.

119. There were 363 (0.5 %) women who reported the use of Echinacea during pregnancy. The most common reasons for Echinacea use were treatment of cold/flu, upper respiratory tract infections (including sinusitis, otitis, tonsillitis, and cough), lower respiratory tract infections (bronchitis and pneumonia), vaginal and oral herpes infections. A total of 206 (0.3 %) and 183 (0.3 %) women had used Echinacea during early and late pregnancy, respectively. Some women had not specified the gestational week of Echinacea use. The dose, preparation and method of administration were not provided. Users of Echinacea were not found to have any increased risk of preterm birth, low birth weight, or small for gestational age. 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 users of Echinacea were hypospadias, cleft lip, and hypoplastic left heart syndrome. (Heitmann et al., 2016).

120. A study conducted over a 10-month period explored the use of herbal products among Italian women at the maternity wards of Padua and Rovereto hospitals (Cuzzolin et al., 2010). A structured anonymous questionnaire was administered to 392 women during face-to-face interviews in two sessions within

3 days of childbirth. During the first session, details about herbal medicine consumption, including preparation, dose, timing of administration, reasons for consumption, level of satisfaction and adverse reactions were collected. In the second session, data bout pregnancy history (smoking, alcohol consumption, morbidities, medication use) and newborn details (gestational age, birth weight, Apgar score, problems at birth, treatments) were collected.

121. One hundred and nine (27.8%) women reported taking one or more herbal remedies during pregnancy with 37.8% of them throughout the entire pregnancy duration. Oral Echinacea was used by 10 (9.2%) of the interviewed women for common cold, anxiety and strengthening of the immune system. No information about the Echinacea species, plant part, type of preparation, dose, duration of intake/trimester is given in the article. By examining each herb separately, the authors report that in one case there was a possible relationship between prolonged Echinacea intake and intrauterine growth restriction in a 35-week newborn. No further detail is provided for that case. (Cuzzolin et al., 2010).

122. A similar study aiming to investigate the use of herbal medicines in pregnant women in relation to pregnancy outcomes involved the administration of a structured questionnaire to 600 women within five days after delivery at Stavanger University Hospital Norway (Nordeng et al., 2011). The women's medical charts were reviewed for information on pregnancy outcomes including birthweight, gestational age, presence of neonatal and/or maternal complications. Forty percent 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 information about the Echinacea species, plant part, type of preparation, dose, duration of intake/trimester is given in the article. Except for birthweight, there were no significant differences between users and non-users of herbal medicines. Mean birth weight was significantly higher (155 g; $p = 0.001$) in the users of herbal medicines (3,663 g) compared to non-users (3,508 g). Sub-analysis of the data revealed that this was linked to the use of iron-rich herbs. The association between mean birth weight and Echinacea in particular is not discussed in the article.

123. The UK Teratology Information Service (UKTIS) has 15 reports of maternal exposure to Echinacea in their database for the period between 2004-2017. Only one of these has a record of the pregnancy outcome. A pregnant woman took Echinacea for a cold between gestational week 7-8 along with several other prescription medicines. The outcome was a normal live-born infant born at gestational week 40. Further details regarding the preparation and dose of

Echinacea were not provided by UKTIS.

Lactation

124. 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.

Adverse effects in humans

125. A meta-analysis of clinical trials (Schapowal et al., 2015) investigating the use of Echinacea supplements in patients with respiratory tract infections (see paragraph 55 for more details on the study) looked at the adverse events recorded in the 6 clinical trials included in the analysis from a total of 1,440 Echinacea-treated subjects and 1,326 subjects receiving placebo. Overall, 491 adverse events occurred with Echinacea in comparison to 474 with placebo, but there were no significant differences between the groups. Most adverse effects reported were gastrointestinal disturbances and were mild and transient. Only two severe adverse events (stridor) occurred with Echinacea and one (glandular fever, requiring hospitalization) in the placebo group. There were no significant differences in clinical biochemistry associated with Echinacea use.

126. Systematic review summarised evidence of the safety of Echinacea based herbal medicinal products from 36 clinical studies, case reports, and spontaneous reporting programmes from regulatory agencies in Australia, Germany, UK, USA and Sweden (Huntley et al., 2005). Their conclusion was that Echinacea has a good safety profile when taken short-term, with short-term use being defined as ‘days as opposed to weeks’. Adverse effects are mild, transient and reversible with gastrointestinal disturbances and skin-related reactions being most commonly reported. The study emphasises that in rare cases Echinacea use can be associated with allergic reactions, which can be severe and cautions against Echinacea use in atopic or asthmatic individuals.

127. The EMA assessment report on E. purpurea (EMA, 2014) concludes that based on the analysis of pharmacovigilance reports from EU member states,

hypersensitivity reactions such as rash, urticaria, itching and swelling are possible and in a case of allergic reaction, Echinacea should not be taken again. There are cases of severe reactions such as Stevens-Johnson Syndrome, angioedema, bronchospasm, asthma and anaphylactic shock with confirmed/probable causality. Cases of autoimmune diseases such as encephalitis disseminata, erythema nodosum, immunothrombocytopenia, Sjögren's syndrome with renal tubular dysfunction have been reported, but the causality of these is inconclusive. The gastrointestinal side effects reported are unlikely to be linked to Echinacea as their frequency is similar in placebo and treatment groups in clinical trials (EMA, 2014).

128. Analysis of spontaneously reported adverse reactions submitted to the Swedish Medical Products Agency from 2007-15 identified 116 reports related to herbal medicinal products (Svedlund et al., 2017). There were 14 reports concerning Echinacea and the most common adverse drug reactions (ADR) were related to skin and subcutaneous tissue (n=7), which could be the result of hypersensitivity or allergy.

129. A systematic review summarised and critically assessed the available data on adverse effects related to plant food supplements (Di Lorenzo et al., 2015). PubMed/MEDLINE and Embase were searched, and the reports were assessed according to the World Health Organization (WHO) Causality Assessment Criteria. A total of 20 papers reporting adverse effects due to Echinacea were identified, but the causality in 10 out of 20 was defined as 'unclassifiable' because there was insufficient evidence of exposure, lack of clear information on the preparation and/or the description of the adverse event. The adverse reactions from papers reporting certain or probable association were either allergy, hepatic or gastrointestinal effects such as diarrhoea, vomiting, headache or drowsiness. Most were associated with preparations containing the ethanolic extracts of herb and root.

130. An Australian study looking at adverse reactions associated with Echinacea identified 51 reports of ADRs in the Australian Adverse Drug Reactions Advisory Committee's database (Mullins and Heddle, 2002). There were 26 cases which were suggestive of IgE-mediated hypersensitivity reactions (4 anaphylaxis, 12 acute asthma,10 urticaria/angioedema). Seventy eight percent of the affected patients were female, median age was 32 years and over half had a history of asthma, allergic rhinitis or atopic dermatitis. In addition, the authors also personally evaluated five privately referred patients in their practice via skin prick testing (SPT) after exposure to Echinacea.

131. Two of these patients suffered anaphylaxis and one had an acute asthma attack after their first ever dose of Echinacea. All three patients were female between the ages of 19 and 37 years, had history of atopy including allergic rhinitis or latex allergy and exhibited a positive SPT to aqueous Echinacea solution when tested in the private practice of the authors (Mullins and Heddle, 2002). One of these cases describes the consumption of 5 mL commercially prepared Echinacea (40% alcohol in water) equivalent to 3,825 mg of whole plant extract E. angustifolia and 150 mg of E. purpurea (Mullins, 1998), whilst the other two involved the use of Echinacea tablets or tea (Huntley et al., 2005; Mullins and Heddle, 2002).

132. The fourth patient was a 56-year-old man who had recurrent episodes of mild asthma each time Echinacea tablets were administered, and the symptoms resolved within a few days of stopping. This patient had allergic rhinitis, but no other drug or medical history. The last case involved a 48-year-old woman who developed a maculopapular rash within 2 days of Echinacea tablets ingestion, and this recurred on rechallenge a week later. This patient had history of no-allergic rhinitis and took no regular medication. Both patients had a negative SPT to aqueous Echinacea solution. (Huntley et al., 2005; Mullins and Heddle, 2002).

133. The authors also tested 100 atopic patients who had never taken Echinacea and 20% had positive STP results. The overall conclusion of the study was that there is a possible cross-reactivity between Echinacea and other environmental allergens and atopic patients should be warned accordingly. (Mullins and Heddle, 2002).

134. An autoimmune disease supposedly triggered by Echinacea was described in a 55-year-old man who had a diagnosis of emphigus vulgaris, which was in remission and was managed with low doses of dapsone. The man started taking daily Echinacea supplements for the treatment of a respiratory tract infection and he developed blisters on his body, head and oral mucosa within 1 week of starting the supplement. After the discontinuation of Echinacea, partial disease control was achieved with prednisolone, azathioprine and dapsone, but never a complete remission. (Lee and Werth, 2004).

135. An isolated case of erythema nodosum was reported in a 41-year-old man who experienced four episodes of erythema nodosum after using Echinacea for flu-like symptoms. He had been using Echinacea intermittently for 18 months alongside loratadine and St. John’s wort. The patient responded to prednisolone treatment, discontinued the Echinacea preparation and after 1 year had not experienced further reoccurrences. (Lee Soon and Crawford, 2001).

136. Hypereosinophilia associated Echinacea use was reported in a 58-year-old male patient (Maskatia and Baker, 2010). The patient complained of mild crampy abdominal pain, with occasional nausea and diarrhoea. He had started taking Echinacea supplements several weeks prior to symptom onset. Past medical history included controlled asthma and allergic rhinitis, hyperlipidaemia, resolved hepatitis B infection, cholelithiasis, an inguinal hernia, and a hiatal hernia. The patient had eosinophil count of 17,800/µL (normal <350 eosinophils/µL) and Immunoglobulin E (IgE) level of 1390 IU/ml (normal <114 IU/m). Serum biochemistry, liver function tests and bone marrow cytogenetics were normal. The patient was treated with prednisolone and stopped taking Echinacea. Two weeks after discontinuing the Echinacea supplements, the eosinophil counts and IgE levels had improved to almost normal levels. Prednisolone was tapered off and six months later, the patient continued to have normal IgE and eosinophil levels.

137. A case of leucopenia was reported in a 51-year-old woman who had a blood test at her annual medical appointment and her white blood count (WBC) had decreased from 5,800/µL the preceding year to 3,300/µL (normal range 4,000 – 11,000/µL). Other than that, she appeared healthy. She had been taking 450 mg Echinacea capsules three times daily for the past 2 months for the prevention of respiratory tract infection as well as Gingko biloba, bupropion, vitamins C, E, B and calcium. She stopped taking Echinacea and her white blood cell count increased to 3,700 µL a month later. The following year, the patient resumed the Echinacea supplement and after two months, her WBC had decreased to 2,800 µL. Once again, she discontinued taking Echinacea and her WBC increased to 4,320 µL 7 months later. The authors of the case report attributed the changes in the white blood cell count to the Echinacea supplements. (Kemp and Franco, 2002).

138. Thrombocytopenia was reported in a 32-year-old man who took oral water–alcoholic extract of the E. pallida for about a week for an upper respiratory tract infection (George et al., 2006). The patient was admitted to hospital 20 days later with hypotension, sinus tachycardia, anaemia, thrombocytopenia, microangiopathic type haemolytic anaemia with fragmented red blood cells, increased indirect bilirubin, and markedly elevated lactate dehydrogenase (LDH) level. Bacterial and viral antibodies were negative. The diagnosis was severe thrombotic thrombocytopenic purpura (TTP) and the patient was treated with transfusion of red blood cells and fresh frozen plasma (FFP). The patient had a syncope episode followed by a seizure and was controlled with general anaesthesia and ventilatory assistance. He was treated with plasmapheresis twice

a day and FFP for over a month until disease remission.

139. Hepatotoxicity associated with Echinacea were reported in the form of severe acute cholestatic autoimmune hepatitis (ACAH) (Kocaman et al., 2008) and fatal liver necrosis (Jacobsson et al., 2009). The fatal liver necrosis was reported in a 28 year old man who took Echinacea 5 days a week for 6 months (Jacobsson et al., 2009). The acute cholestatic autoimmune hepatitis (ACAH) occurred in a 45-year-old male patient who took Echinacea root at 1,500 mg/day for the treatment of a cold (Kocaman et al., 2008). The patient had fatigue and jaundice with elevated alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and immunoglobulin G (IgG). Anti-smooth muscle antibodies (ASMAs) were positive, whilst anti-nuclear antibodies, anti-liver/kidney microsomes, anti-soluble liver antigen antibodies, and anti-mitochondrial antibodies were negative. Liver biopsy revealed hepatitis, prominent cholestasis, portal lymphoplasmocytic and eosinophilic granulocyte infiltration. Echinacea supplementation was discontinued and a month later all laboratory values were normal except SMA positivity. The authors concluded that this was a case of Echinacea-induced ACAH due to the immunostimulatory effects of Echinacea.

Duration of use

140. EMA recommends that oral Echinacea preparations should be used for a limited duration of up to 10 days (EMA, 2014). The German Commission E monographs on Echinacea recommend that internal and external administration of E. purpurea and E. pallida should not exceed 8 weeks (Blumenthal et al., 1999). No scientific rationale has been provided for the limits on the duration of use. Echinacea preparations have been used for longer durations without any serious adverse effects as described below.

141. The clinical studies involving Echinacea have varying durations from 4-21 days to 4-12 weeks (Ardjomand-Woelkart and Bauer, 2015). The study with the longest duration involved the administration of 800 mg E. purpurea whole plant extract twice a day for 6 months to 50 patients (Vonau et al., 2001). The only side effects reported were nausea (n = 4) and diarrhoea (n = 2). The use of E. purpurea and E. angustifolia root liquid extract for 12 weeks (100 drops daily of a 1:11, 30% ethanolic extract for 5 days a week) was studied in randomized, double-blind, placebo controlled trial involving 289 patients for the prevention of respiratory tract infections (Melchart, 1998). The side effects reported included minor gastrointestinal symptoms, headache/dizziness, allergic reactions and were

similar between treatment arm and placebo.

142. The safety and efficacy of Echinaforce was tested in a large randomised, double-blind, placebo-controlled clinical trial for 4 months. A total of 755 subjects were included and the main criteria for inclusion was that they experience ≥2 colds per year. Participants took the equivalent of 2,400 mg of extract a day for illness prevention, but during acute stages of colds the dose was increased to 4,000 mg extract/day. There were no significant differences between the frequencies and the type of adverse effects between treatment and placebo. Haematological and biochemical measures were not significantly different before and after Echinacea treatment and when compared to placebo (Jawad et al., 2012).