Toxicity Studies

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Cytotoxicity

E. purpurea

47. Tsai et al. (2012a) investigated the cytotoxicity of E. purpurea flower extract and its bioactive constituent chicoric acid in human colorectal cancer cell lines (HCT-116 and Caco-2). Treatment with Echinacea extract (0 – 2,000 µg/mL) for 24 hours did not affect cell viability, but a dose-dependent reduction was observed at 48 hours. Chicoric acid significantly decreased cell viability at ≥150 µg/mL after 24 hours and at all tested concentrations (50–200 µg/mL) after 48 hours. In HCT-116 cells, chicoric acid (50–150 µg/mL) suppressed telomerase activity, induced DNA fragmentation, activated caspase-9, and promoted PARP cleavage, indicating apoptosis. 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.

E. angustifolia

48. The cytotoxicity of ethyl acetate extract of E. angustifolia 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 3-4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay (Espinosa-Paredes et al., 2021). The extract contained 11.2 µg/mg echinacoside and 8.18 µg/mg caffeic acid. The study reported that the E. angustifolia extract showed no cytotoxicity toward the healthy MCF 10 cells, whereas it reduced cell viability in both cancer cell lines, with calculated IC_{50} values between 16.3 and 28.8 µg/mL and no significant differences between 24 h and 48 h time points. The study also concluded that the E. angustifolia extract induced cell cycle arrest in the G1 phase and caspase-mediated apoptosis.

Acute toxicity

E. purpurea

49. A single high dose study reported no deaths or signs of toxicity following oral or intravenous administration of E. purpurea expressed juice to Wistar rats and NMRI mice (Mengs et al., 1991). No abnormalities were seen during a 14 day observation period or during necropsy, and an LD_{50} could not be determined.

E. angustifolia

50. A single dose study, compliant with the Organisation for Economic Co-operation and Development test guideline (OECD TG) 432, reported no clinical signs, mortality, or macroscopic lesions after the administration of 2,000 mg/kg

bw of E. angustifolia ethyl acetate extract to three male CD-1 mice (Espinosa-Paredes et al., 2021). The extract contained 11.2 µg/mg echinacoside and 8.18 µg/mg caffeic acid. The authors classified the LD50 of the E. angustifolia extract as Category 5 under the Globally Harmonized System (GHS) (>2,000–5,000 mg/kg), indicating very low acute toxicity.

Sub-acute toxicity

E. purpurea

51. Expressed juice from E. purpurea was administered via oral gavage to groups of 18 Wistar rats per sex at doses of 0, 800, 2,400, or 8,000 mg/kg body weight daily for four weeks (Mengs et al., 1991). A statistically significant reduction in plasma alkaline phosphatase was observed in males at 2,400 and 8,000 mg/kg, while females exhibited a significant increase in prothrombin time at the same dose levels 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 study reported that all other parameters, including biochemical and haematological results, body weight, food consumption, ophthalmological findings, necropsy, and histopathology, showed no significant differences among treatment groups. The authors stated compliance with the OECD GLP principles and to OECD recommendations for technical methods, although no specific OECD TG applicable to the subacute toxicity study was referenced.

E. angustifolia

52. Espinosa-Paredes et al. (2021) conducted a 28-day repeated-dose toxicity study with ethyl acetate extract of E. angustifolia. The extract, containing 11.2 µg/mg echinacoside and 8.18 µg/mg caffeic acid, was administered to five CD-1 mice per dose per sex at 20 mg/kg bw or 200 mg/kg bw in accordance with the OECD TG 407. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine levels were determined. No statistically significant differences were observed between treated and control groups, and the authors concluded that there was no evidence of liver or kidney toxicity associated with the E. angustifolia extract.

Sub-chronic toxicity

E. purpurea

53. 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 study was conducted in accordance with Good Laboratory Practice (GLP) regulations and Test Guidelines of Standards for Toxicity Studies of Drugs issued by the Korean Food and Drug Administration. The E. purpurea extract, standardised to contain at least 2% chicoric acid, was administered daily at doses of 0, 500, 1,000, and 2,000 mg/kg body weight to groups of 10 rats per sex. No mortality or abnormal clinical signs were observed in either sex at any of the tested doses. The study reported that the ophthalmological examinations, absolute and relative organ weights, haematology, and serum biochemistry showed no significant differences between treated and control groups. The urinalysis revealed a statistically significant increase in mean urine volume in males at 1,000 mg/kg compared to controls. Some individual variations were also observed in the urinalysis, but the study concluded they were not significantly different when compared to the controls.

Genotoxicity

E. purpurea

In vitro genotoxicity assays

Bacterial reverse mutation tests

54. A lyophilised Echinacin Liquidum herbal medicinal product (E. purpurea) was evaluated for mutagenic potential in an in vitro bacterial reverse mutation assay using Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, with and without S9 metabolic activation, at concentrations ranging from 8 to 5,000 µg/plate (Mengs et al., 1991). The test material, the bacterial strains and S9 mix were incorporated into molten agar, poured onto minimal agar plates and incubated at 37°C for three days. The authors reported that across three replicates, mean revertant counts showed no reproducible, statistically significant or dose related increases in any strain, with or without metabolic activation. Since no positive response was observed at any concentration up to the limit dose of 5,000 µg/plate, the study concluded that the tested product exhibited no mutagenic potential. The study stated compliance with OECD GLP principles and OECD technical recommendations, although no specific OECD TG applicable to the bacterial reverse mutation test was referenced.

55. The mutagenicity and the antimutagenic effects of freeze dried E. purpurea 50% ethanolic extracts were evaluated in S. typhimurium TA98 and TA100 strains with and without S9 metabolic activation at a maximum concentration of 5,000 µg/plate (Tsai et al., 2012b). The ethanolic extracts contained caftaric acid, chlorogenic acid, echinacoside and cichoric acid, but no cynarin. For the mutagenicity assay, the bacteria were mixed with 250-5,000 µg/plate of the extract, incubated on histidine containing media at 37°C for 48 h and revertant colonies were compared with vehicle controls. The study reported that the E. purpurea extract did not induce any significant increases in revertant numbers in either strain, at any concentration under any condition, when compared to control plates treated with dimethyl sulfoxide (DMSO). The authors also assessed antimutagenic activity of the extract by calculating the percentage inhibition of the reversion rate in the presence of 2 aminoanthracene and reported that the E. purpurea extract demonstrated a dose dependent inhibitory effect on 2 aminoanthracene induced mutagenicity in both S. typhimurium strains. There was no indication as to whether the bacterial reverse mutation test had been conducted in compliance with OECD TG.

56. E. purpurea extract standardised to contain at least 2% chicoric acid was tested in an in vitro bacterial reverse mutation test in S. typhimurium strains TA98, TA100, TA1535 and TA1537 and tryptophan auxotroph mutant Escherichia coli strain (WP2uvrA) in accordance with OECD TG 471 (Jeong et al., 2024). The bacterial strains were incubated with the E. purpurea extract at up to 5,000 µg/plate with and without S9 mix, for 48 hours at 37°C. 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 authors stated that the number of mutant colonies at all doses of each strain did not exceed twice that of the negative control and results were considered negative for mutagenicity.

Mouse lymphoma assay

57. A lyophilised Echinacin Liquidum herbal medicinal product (E. purpurea) was evaluated for its potential to induce mutations at the hypoxanthine phosphoribosyltransferase (HPRT) locus of L5178Y mouse lymphoma cells, both in the presence and absence of S9 metabolic activation, at concentrations ranging from 50 to 5,000 µg/mL (Mengs et al., 1991). The cells were exposed to the test or control solution for 2 hours, then allowed a 7 day expression period before plating with and without 6 thioguanine to determine mutation frequency. A response was considered significant only if treated cultures exceeded the 95th

percentile threshold of controls, occurred at consecutive doses, and showed a dose response by regression analysis. Cytotoxicity was assessed via plating efficiency immediately after treatment. The study reported that the Echinacea treatment did not produce a statistically significant increase in mutation frequency at any of the concentrations tested, with and without S9 metabolic activation, and that doses up to 5,000 µg/mL were considered essentially non toxic. The study stated compliance with the OECD GLP principles and OECD technical recommendations, although no specific OECD TG applicable to the mouse lymphoma assay were referenced.

Chromosomal aberration tests

58. Mengs et al. (1991) also tested the lyophilised Echinacin Liquidum herbal medicinal product (E. purpurea) in an in vitro cytogenetic assay using human lymphocyte cultures, with and without S9 metabolic activation, at concentrations ranging from 2,400 to 5,000 µg/mL. The cells were exposed to the test or control solution for either 20 or 44 h (without S9) or for 3 h followed by recovery (with S9), then analysed for mitotic index and chromosomal aberrations. The authors stated that no mitotic inhibition was observed at any concentration under any condition. A small but statistically significant increase in the proportion of cells with structural chromosomal aberrations was reported at 5,000 µg/mL at the 20 h sampling without S9. However, the authors considered this biologically insignificant because values remained within the laboratory’s normal range and coincided with increased osmolality of the treatment medium. The study stated compliance with OECD GLP principles and OECD technical recommendations, although no specific OECD TG applicable to the chromosome aberration assay were referenced.

59. E. purpurea extract standardised to contain at least 2% chicoric acid was tested in an in vitro chromosomal aberration test using Chinese Hamster Lung (CHL/IU) cells in accordance with OECD TG 473 (Jeong et al., 2024). The cells were incubated with the E. purpurea extract at concentrations of 78.1, 156 and 313 µg/mL, in the presence or absence of S9, for either 6 or 24 hours for the short-term and continuous treatment, respectively. Mitomycin C at a concentration of 0.1 µg/mL and benzo[a]pyrene at 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. The study reported that no statistically significant increase in structural or numerical chromosomal aberrations was observed in the Echinacea treated cells compared to controls at any of the

concentrations tested, with and without S9 metabolic activation.

In vivo micronucleus test

60. Mengs et al. (1991) conducted an in vivo micronucleus test using a single oral dose of 25,000 mg/kg Echinacin Liquidum (E. purpurea) via oral gavage to groups of 5 male and 5 female NMRI mice. The animals were sacrificed at either 24, 48 or 72 hours after the dose. Positive control groups received 100 mg/kg cyclophosphamide via intraperitoneal injection, and negative controls received 25 mL/kg water by oral gavage. Bone marrow was collected from the femur, and two smears per animal were prepared, fixed, and stained. One thousand polychromatic erythrocytes (PCE) per animal were scored microscopically for micronuclei. The authors reported that 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. The PCE/NCE ratio was also not significantly different between treated and control groups. The study stated compliance with OECD GLP principles and applicable OECD technical recommendations, although it did not reference a specific OECD TG for the in vivo micronucleus test. Additionally, no toxicokinetic measurements were reported to demonstrate bone marrow exposure to the test substance.

61. E. purpurea extract standardised to contain at least 2% chicoric acid was orally administered at 1,250 and 5,000 mg/kg bw to seven-week-old male Sprague Dawley rats (5 animals per dose) in a vivo micronucleus test in accordance with OECD TG 474 (Jeong et al., 2024). Positive control groups received 20 mg/kg cyclophosphamide orally, and negative controls received water. Following the second dose, bone marrow was collected from the femurs, fixed, and stained with acridine orange for fluorescence microscopy. Genotoxicity was assessed by counting micronucleated polychromatic erythrocytes (MNPCE) among 4,000 PCE per rat, and cytotoxicity was evaluated based on the proportion of PCE among 500 red blood cells. Cytotoxicity indices were comparable across all groups. The study reported that the positive control produced a genotoxicity index approximately 100-fold higher than the negative control, whilst none of the Echinacea dose groups showed statistically significant differences in genotoxicity relative to the negative control. The authors also conducted a toxicokinetic study with pure chicoric acid and the standardised E. purpurea extract and noted that the pharmacokinetics of chicoric acid were comparable between the purified compound and the standardised E. purpurea extract, with similar simulated concentration–time profiles volume of distribution and clearance.

E. angustifolia

62. Espinosa-Paredes et al. (2021) conducted an in vitro bacterial reverse mutation test and an in vivo micronucleus test with E. angustifolia ethyl acetate extract containing 11.2 µg/mg echinacoside and 8.18 µg/mg caffeic acid. There is no indication as to whether these tests had been performed in accordance with the OECD TG.

63. For the bacterial reverse mutation assay, the bacterial strains S. typhimurium TA98, TA100 and TA102 were incubated with the E. angustifolia extract at up to 200 µg/plate, with and without S9 mix, for 48 hours at 37°C. Picrolonic acid, 2-amino-anthracene, N-methyl-N′-nitro-N-nitrosoguanidine and mitomycin-C were used as a positive control, whilst 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 result and concluded no genotoxic activity was therefore observed.

64. For the in vivo micronucleus test, the E. angustifolia extract was administered intragastrically at 1,000 mg/kg bw to three male CD-1 mice. 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 frequencies of normochromatic erythrocytes (NCEs) and reticulocytes (RETs), with and without micronuclei (MNs), were evaluated in order to calculate the percentages of mature normochromatic erythrocytes (% MN-NCEs), micronucleated reticulocytes (% MN-RETs) and total reticulocytes (% RETs). The study reported that the E. angustifolia extract did not induce a significant increase in micronuclei formation, with %MN-RET at 0.9% compared to 0.3% in the negative control. A decrease in the frequency of RET in the Echinacea extract group compared with the negative control (2.56% vs 5.41%, $p < 0.05$) was reported, but the authors did not comment on its biological relevance. No toxicokinetic measurements were reported to demonstrate bone marrow exposure to the test substance.