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A REVIEW ON PHARMACOLOGICAL AND THERAPEUTIC PROPERTIES OF *ECHINACEA*

Mostafa Ganjuri [1], Sara Darakhshan [* 2, 3] and Fereidoun Taghizad [4]

Department of Biology [1], Faculty of Science, Department of Physiology [4], Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
Department of Biology [2], Faculty of Science, Razi University, Kermanshah, Iran.
Department of Pharmaceutics [3], Faculty of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.

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Correspondence to Author:
Sara Darakhshan

Department of Biology,
Faculty of Science, Razi University,
Kermanshah, Iran.

E-mail: darakhshan.sara@gmail.com

ABSTRACT: The botanical supplement market is growing at a rapid rate, and this trend is expected to continue to progress. In the world of Nutraceuticals, *Echinacea* plant is widely used for medicinal and commercial purposes. This Native American herb has a remarkable record of clinical and laboratory study, as well a long history of medicinal use in the management of a variety of conditions. Phytomedicinal preparations from the genus of *Echinacea* are widely used for the prevention and the treatment of common cold and upper respiratory tract infections. However, most of the uses of *Echinacea* are based on the reported immunological properties; there is a large body of evidence, based on *in-vitro* and animal studies, demonstrating that *Echinacea* possesses anti-inflammatory, anti-oxidative, and anti-microbial properties. It has also been suggested that this plant is a potential therapeutic agent for cancer, diabetes and skin problems. From the other point of view, by the available safety data, *Echinacea* has little adverse effects and is well tolerated. This paper reviews the pharmacological properties of the *Echinacea* genus and its active components.

INTRODUCTION: Nowadays, the use of herbal/ botanical products has gained growing acceptance by the public due to the belief that they are natural and, therefore, safe [1]. *Echinacea* has become a best-selling medicinal herbal preparation of all time in North America and Europe [2]. Several species in the genus of *Echinacea* has been used for centuries, customarily as a remedy to treat a number of ailments including common cold, coughs, bronchitis, upper respiratory infections (URI), abscesses, wound infections, gangrene, eczema, dizziness, sore eyes, snake bites, syphilis, typhoid, malaria, diphtheria, hemorrhoids, and tumors [3, 4].

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Numerous pharmacological investigations are available in literature, and they are related to several kinds of preparations obtained from the species of *Echinacea*. Interest in *Echinacea* is focused on its immunomodulatory effects [5-9], and particularly several clinical trials have been done in the prevention and treatment of common cold and URIs [10-14].

The beneficial effects of *Echinacea* are also in a variety of disease states, such as inflammation [15, 16], oxidative conditions [17, 18], cancer [19-21], skin problems [22], and liver diseases [23]. Also, *Echinacea* exhibits anti-microbial [24-27] and antidiabetic properties [28-30]. This review focuses on the pharmacological benefits of *Echinacea* and its active ingredients in the context of its therapeutic potentials **Fig. 1.** All of the relevant databases were searched for the term "*Echinacea*" and information on *Echinacea* was collected via electronic search by using PubMed and Science Direct.

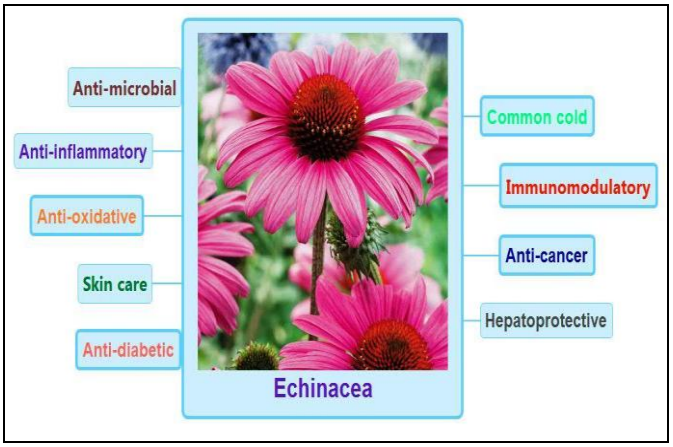

FIG. 1: PHARMACOLOGICAL PROPERTIES OF *ECHINACEA* PLANT

Characteristics and Chemical Compounds: *Echinacea*, commonly called coneflower, is a genus of herbaceous perennial flowering plants in the daisy family, Asteraceae and originates in eastern North America [2]. Traditional taxonomic system has reported that nine species of *Echinacea* exist in nature [31] **Table 1**, however, under a recent reclassification system, four species and eight varieties are categorized [32]. Among species of the *Echinacea* genus, *E. purpurea*, *E. angustifolia*, and *E. pallida* have been the most widely used in medicine for their pharmacological properties [33] **Fig. 2**.

TABLE 1: SCIENTIFIC CLASSIFICATION OF *ECHINACEA*

Kingdom	Plantae
Order	Asterales
Family	Asteraceae (Compositae)
Genus	*Echinacea*
Species	*Angustifolia, atrorubens, Laevigata, pallida, paradoxa, purpurea, sanguinea, simulata, tennesseensis*

FIG. 2: a) *ECHINACEA PURPUREA*, b) *E. ANGUSTIFOLIA* AND c) *E. PALLIDA*

The main bioactive compounds present in *Echinacea* species include alkylamides (alkamides), caffeic acid and its derivatives (caftaric, chlorogenic and cichoric acid, cynarin and echinacoside), polysaccharides, glycoproteins, lipoproteins, and acetylenes (polyacetylenes and polyenes) [34, 35]. There are trace elements including Ca, Mg, Fe, Mn, Cu, Zn, Ni, and Li in root, stem, leaves, and flowers [36]. However, the concentration of key compounds in species of *Echinacea* varies by plant age, plant parts used, growth conditions, geographical location, and method of extraction [37].

Also, the distribution of chemical compounds and biological activities differ within the same plant (root, stem, leaves, and flowers) [38]. For example, the alkylamide content in *Echinacea* species appears to be higher in roots, to increase with age and to differ with the geographical area of cultivation [38].

Several forms of commercial preparations are available that use different parts of the *Echinacea* plant such as roots, seeds, flowers, and leaves. The most commonly used form is the tincture, which is a liquid ethanol-water extract. Other forms include powdered ethanol-water extract, freeze-dried ethanolic or hydrophilic extracts, capsule and tablet, skin cream, ointment, and gel, and pressed juice and tea.

Anti-inflammatory Effects: Currently special emphasis has been given to the role of inflammation in the pathogenesis of diseases. For a long time, medicinal plants have been used as remedies for various inflammatory conditions. The alcoholic extracts of *E. angustifolia*, *E. purpurea*, and *E. pallida* significantly inhibited nitric oxide (NO) production by lipopolysaccharide (LPS)-activated the RAW 264.7 macrophage cells, among which *E. pallida* was the most active.

The enzymes nitric oxide synthase (iNOS) and arginase metabolize a common substrate, L-arginine, but produce different biological effects. While iNOS is involved in inflammation, arginase contributes to an anti-inflammatory response. *Echinacea* can modulate the iNOS/arginase balance due to anti-inflammatory activation.

The alcoholic extract of *E. pallida* inhibited iNOS enzyme at the protein level in LPS-treated RAW 264.7 macrophages, whereas arginase activity of the cells was significantly improved by alcoholic

extracts of *E. angustifolia*, *E. pallida* and *E. purpurea*. The hydrophilic fraction containing caffeic acid derivatives enhanced arginase activity, while the hydrophobic fraction containing alkamides inhibited iNOS expression and NO production [15]. The *Echinacea*-mediated decrease in NO production was generally associated with a decreased protein level of iNOS, *Echinacea* present at the onset of LPS-induced iNOS expression led to a stronger inhibitory effect on NO production.

An alcoholic tincture of *E. purpurea* roots considerably inhibited the nuclear expression of pro-inflammatory transcription factors NF-κB and STATs in rhinovirus-infected cells [39]. In a study using gene and protein array analysis, the effects of *Echinacea* commercial preparations (E1 was an aqueous expressed juice of the aerial parts of *E. purpurea*, and E2 was a 50% ethanol tincture from *E. purpurea* roots) on rhinovirus infection was evaluated by BEAS-2B, a line of human tracheobronchial epithelial cells.

It found that rhinovirus infection would increase some inflammatory cytokines at mRNA and protein levels and that these effects could be reversed by *E. purpurea*. Substantial increases were observed about the pro-inflammatory cytokines IL-6 and IL-8 (CXCL8) at protein levels that was ameliorated by *E. purpurea* [40].

E. angustifolia preparation enriched fractions Bauer alkylamide 11 and Bauer ketone 23 showed anti-inflammatory effects in LPS-induced RAW264.7 mouse macrophage cell line. These treatments decreased prostaglandin (PG)E2 production and inhibition of PGE2 production may be due to the targeting of cyclooxygenase (COX)-2 enzyme activity [41]. In macrophages subjected to hydrogen peroxide (H2O2), treatment by *E. angustifolia* extract reversed mRNA expression of COX-2, interleukin (IL)1-β, NF-κB1, NF-κB2, and tumor necrosis factor (TNF)-α. Also peroxisome proliferator-activated receptor (PPAR)-γ to normal levels [42]. In a study, 50% ethanolic tinctures of *E. tennesseensis* were prepared from roots, stems, leaves, and flowers of the plant. Fresh root, leaf, and flower tinctures stimulated human peripheral blood mononuclear cells (PBMC) proliferation *in-vitro*, also fresh root tincture stimulated IL-10 production [43]. Oral administration of *Echinacea* alcoholic extract to mice increased production of anti-inflammatory cytokines IL-4 and IL-10 but decreased the production of TNF-α and IL-1β in activated spleen cells [44].

In murine macrophages, the alkamides significantly inhibited COX-2 activity and suppressed the LPS-induced expression of COX-2, iNOS, TNF-α, IL-1α, IL-6, and monocyte chemotactic protein (MCP)-1, but elevated heme oxygenase-1 (HO-1) protein expression [16]. In RAW264.7 macrophage cells, chemically synthesized Bauer Ketones 21 and 23 from *E. pallida* each significantly inhibited both PGE2 and NO production, as well Bauer Alkylamide 11 repressed production of PGE2 and NO [45]. Other results showed that polyunsaturated alkamides, undeca-2Z-ene-8,10-diynoic acid isobutylamide (A5), dodeca-2E-ene-8,10-diynoic acid isobutylamide (A7), and dodeca-2E,4Z-diene 8,10-diynoic acid 2-methylbutylamide (A8), isolated from roots of *E. angustifolia* were efficient inhibitors of COX-2 activity and suppressed PGE2 formation in H4 human neuroglioma cells [46]. Ethanolic extract of *E. paradoxa* var. paradoxa, rich in polyenes/polyacetylenes suppressed LPS-induced production of NO, PGE2, IL-1β and IL-6 in stimulated RAW264.7 macrophage cells. Pentadeca-8Z-ene-11, 13-diyn-2-one (Bauer ketone 23) and pentadeca-8Z, 13Z-dien-11-yn- 2-one (Bauer ketone 24) from *E. paradoxa* were found mainly responsible for inhibitory effects on NO and PGE2 production [47].

A polysaccharide from water extract of *E. purpurea* roots inhibited Pam3Csk4-stimulated release of TNF-α by human THP-1 acute monocytic leukemia cells. This anti-inflammatory activity was shown to be mediated by the phosphatidylinositol-3-kinase (PI3K)/Akt signaling pathway [48]. The cichoric acid from *E. purpurea* extract has anti-inflammatory activity in rheumatoid arthritis by collagen-induced arthritis rat model.

Administration of cichoric acid for 4 weeks significantly decreased the paw swelling, restored body weight gain, and decreased the organ index of the thymus and spleen compared with that of the arthritis group. The cichoric acid reduced the TNF-α, IL-1β and PGE-2 levels in serum; also decreased the levels of NF-κB, TNF-α and COX-2 in synovium tissues of the ankle joint compared with

the arthritis group [49]. A polysaccharide fraction obtained from *E. angustifolia* root extract (0.5 mg/kg) inhibited the carrageenan-induced rat paw edema in intravenous injection and the croton oil-induced mouse ear dermatitis when applied topically. Polysaccharidic fraction also reduced the leukocytic infiltration of the croton oil dermatitis, evaluated both from histological and peroxidase activity aspects [50]. These studies suggesting that *Echinacea* influences inflammatory pathways and may modulate the pro/anti-inflammatory ratio.

***Echinacea* as an anti-oxidant**: The relationship between free radicals and the incidence of various types of diseases has led to considerable interest in preventive medicine in assessing the free radical scavenging activity of medicinal plants and other nutritional anti-oxidant supplements. There have been several reports on anti-oxidative benefits of *Echinacea* species.

Methanolic extracts of freeze-dried *E. purpurea*, *E. angustifolia* and *E. pallida* roots have anti-oxidant activities and were capable of scavenging hydroxyl radical (OH·). Similar scavenging capacities for each extract were found for both 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and ABTS radical. Moreover, all three species were found to delay the formation of conjugated diene hydroperoxide-induced by the thermal decomposition of 2, 2'-azobis (2-amidinopropane) dihydrochloride and extend the lag phase of peroxidation of soybean liposomes. Each root extract suppressed the oxidation of human low-density lipoprotein (LDL), following oxidative modification by Cu^{2+} [17]. The efficacy of the extracts from the stems, leaves, and roots of *E. purpurea* in the reaction with DPPH correlated well with the amount of cichoric acid present in the extracts. The alkamides from *E. purpurea* alone showed no anti-oxidant effect; however, alkamides present in the extract improved the anti-oxidative activity of cichoric acid in the peroxidation lipid emulsion [51].

In another study, the antioxidant activities of echinacoside and caffeic acid were compared by measuring their inhibition of Cu^{2+}- catalyzed oxidation of human LDL. The efficiency of antioxidant activity of the tested substances was: cichoric acid had the highest capacity; the second was echinacoside, and finally caffeic acid. Synergistic anti-oxidative effects of *Echinacea* components were found when cichoric acid (major caffeic acid derivative in *E. purpurea*) or echinacoside (major caffeic acid derivative in *E. pallida* and *E. angustifolia*) were combined with a mixture of alkamides and an aqueous extract containing the high molecular weight polysaccharides [52].

E. purpurea was given to mice at a dose of 30 mg/kg body weight for 14 days before and after irradiation with 3 Gy of γ-rays. The results reflected the reduced effects of γ-rays on peripheral blood hemoglobin and red blood cells, differential white blood cells and bone marrow cells counts in γ-irradiated mice. The changes observed in thiobarbituric acid-reactive substances (TBARs) level, superoxide dismutase (SOD) and glutathione peroxidase (GSPx) activities, as well DNA fragmentation were ameliorated by *E. purpurea* administration [18]. In a study, the radio-protective properties of *E. purpurea* tablets in a group of radiation workers who were identified as carrying dicentric chromosomes in their lymphocytes were investigated. All radiation workers were taking two 275 mg *Echinacea* tablets for two-week treatment. At the end of the period, lymphocyte chromosome aberrations frequency dropped significantly, and the number of apoptotic cells increased [53].

E. purpurea administration had a suppressive effect on radiation-induced lymphocytopenia and monocytopenia and resulted in a faster recovery of blood cell counts. *E. purpurea* activated macrophages to stimulate IFN-γ production in association with the secondary activation of T lymphocytes, and cytokines released from macrophages in mouse peripheral blood after *E. purpurea* administration activated helper T cells to proliferate. Also, SOD activity in peripheral blood was increased because of echinacoside and caffeic acid as anti-oxidants which eliminate superoxide ($O2^{-}$) [54]. These results indicate that *Echinacea* and its derivatives are a good source of natural anti-oxidants and could be used to prevent the damaging effects of free radicals.

Immunomodulatory Activities: Herbal medicine provides several remedies for strengthening the body's resistance to illness by acting on various components of the immune system. A class of

medicinal plants, known as immunomodulators, alters the activity of immune function through the regulation of molecules such as cytokines, chemokines, and immunoglobulins. *Echinacea* is a wide-spectrum immunomodulator that regulates both innate and adaptive immune responses.

In normal rats, oral administration of *Echinacea* preparations (containing its components cichoric acid, polysaccharides, and alkylamides) two times/day for 4 days improved phagocytic activity of alveolar macrophage, also TNF-α and NO release by the LPS-stimulated alveolar macrophages. An enhanced release of TNF-α and IFN-γ in response to *Echinacea* was apparent in spleen macrophages [55]. In a study, extract of *Echinacea* aerial parts (50 mg/kg) was administered over 8 weeks to male Sprague-Dawley rats.

This treatment significantly increased circulating total white cell counts, and IL-2 level [56]. Normal human peripheral blood macrophages cultured in the presence of *Echinacea* ethanolic extract produced higher amounts of IL-1, TNF-α, IL-6, and IL-10 than unstimulated cells. *E. purpurea* increased the number of cytotoxic T cells and suppressor T cells. An increase was observed in IFN-γ levels which activated cell-mediated immune responses, such as proliferation and activation of type I helper T (Th1) cells [57].

Sullivan *et al*., examined the effects of polysaccharides isolated from *E. purpurea* on macrophages. Murine peritoneal macrophages were cultured with *E. purpurea* extract enriched for polysaccharide. The results showed an *E. purpurea* extract stimulated production of IL-6, IL-12, and NO from macrophages.

E. purpurea triggered a signaling cascade within macrophages through both TLR4-dependent and -independent mechanisms, involving ERK, p38 and c-Jun N-terminal kinase (JNK), and ultimately the activation of NF-κB [58]. *E. purpurea* whole herb and root powders were found to stimulate murine macrophage for TNF-α, IL-1α, IL-1β, IL-6, IL-10, and NO secretion, as well as to enhance the viability significantly and proliferation of human peripheral blood mononuclear cells (PBMCs) *in-vitro* [59].

Randolph et al. in a preliminary study in six healthy individuals found that *in-vitro* exposure of THP-1 peripheral leukocytes to *Echinacea* extract induced expression of the IL-1α, IL-1β, IL-8, TNF-α, intracellular adhesion molecule (ICAM), and IL-10 genes. Serum and hematological values were not different from baseline, suggesting that liver or bone marrow responses were not involved in acute responses to *Echinacea* [60]. Echinaforce® is the standardized *E. purpurea* preparation from the combined root and aerial ethanolic extract, from Bioforce, Switzerland. In an *ex-vivo* study oral administration, Echinaforce® reduced the pro-inflammatory mediators TNF-α and IL-1β, and increased anti-inflammatory IL-10 levels.

Chemokines macrophage inflammatory protein-1 (MIP-1)α and IL-8 were up-regulated in blood samples from subjects treated with Echinaforce® [61]. Polinacea® is a standardized hydroethanolic extract obtained from *E. angustifolia* roots containing echinacoside, the high molecular weight polysaccharide IDN 5405 and an isobutylamide fraction. Polinacea® in 10 healthy subjects was administered as herbal syrup once a day for 30 days (containing 100 mg). Results showed the up-regulation of mRNA expression of IL-2 and IL-8 and the down-regulation mRNA levels of the pro-inflammatory cytokines TNF-α and IL-6; these changes positively correlated with the protein levels detected in the plasma [62]. *E. purpurea* ethanolic extract can regulate the differentiation of dendritic cells (DCs), which are known as professional antigen-presenting cells. Alkylamide-rich root extract similarly promoted maturation of human DCs as LPS; however, the stem plus leaf extract inhibited DC maturation. Down-regulation the mRNA expression of chemokines MIP-1α, and MCP-2, and receptors CD191 (CCR1) and CDw199 (CCR9) were observed in stem plus leaf extract-treated DCs.

Regulatory molecules MIP-1β and MCP-1 involved in the c-Jun pathway were found to be up-regulated in root extract-treated DCs [5]. The polysaccharide-rich root extract of *E. purpurea* increased the expression of MHC class II, CD86, and CD54 surface biomarkers after 48 h of exposure, whereas the alkylamide-rich leaf extract inhibited the expression of these molecules. Production of IL-6 and TNF-α increased with exposure to the root; in

contrast, the leaf extract inhibited COX-2 activity. While both extracts decreased the uptake of ovalbumin by murine bone marrow-derived dendritic cells (BMDCs), the leaf extract inhibited the antigen-specific activation of naïve CD4+ T cells from OT II/Thy1.1 mice [7].

Echinacea is found to be a potent activator of natural killer (NK) cells; these cells are active in non-specific immunity against virus-mediated infections and tumors. Mice immunized with sheep red blood cells (sRBC) were gavaged for 7 days with each of the alcohol extracts from *E. angustifolia, E. pallida,* and *E. purpurea*. The three extracts increased percentages of CD49+ and CD19+ lymphocytes in spleen and NK cell cytotoxicity.

Antibody response to sRBC was improved equally by extracts of all three *Echinacea* species. Also, each extract significantly increased IFN-γ production but inhibited the release of TNF-α and IL-1β. *E. angustifolia*- and *E. pallida*-treated mice demonstrated higher production of IL-4 and increased IL-10 production [44]. *E. purpurea* may be capable of stimulating non-specific immunity in the elderly.

E. purpurea after two weeks of administration could increase the number of NK cells in aging mice, reflecting increased new NK cell production in their bone marrow generation site, leading to an increase in the absolute numbers of NK cells in the spleen, their primary origin [63].

Administering root extract of *E. purpurea* daily for 50 days from the onset of leukemia in mice augmented NK cells and prolonged life span. Treatment had an increasing effect on the absolute numbers of NK cells in spleens in 9 days (intermediate stage leukemia). Three months after leukemia beginning, *E. purpurea*-treated leukemic mice had 2-3 times the normal numbers of NK cells in their spleens, also all the major hemopoietic and immune cell lineages in their bone marrow origin site were recorded at normal numbers [64].

Echinacea increased the frequency of NK target conjugates and activated the programming for lysis of NK cells. *Echinacea* resulted in the activation of CD69 expression and an increase in mean fluorescence intensity in both the CD16+ and CD56+ NK subsets. As well, the frequency of CD56+ killer cells in the conjugates was also significantly increased by *Echinacea*. There was the recruitment of non-conjugated CD56+ cells into CD16+ NK-target conjugates and activation of the NK-target non-killer conjugates into killer cells [65].

Water-soluble extract from *E. purpurea* fresh aerial parts (containing 80% polysaccharides, phenolic compounds, cynarin, cichoric and caftaric acids) strongly enhanced T-cell production of IL-2 and IFN-γ in response to phorbol 12-myristate 13-acetate (PMA) plus ionomycin stimulation [66]. The addition of cichoric acid from a root extract (prepared from both *E. angustifolia* and *E. purpurea*) and 2,4-diene alkylamide-derived from combination of both species induced NF-κB expression after PMA stimulation of Jurkat T-cell line [67].

In a study by Sasagawa *et al.*, Jurkat cells were treated with ethanolic extract prepared from dried leaves and flowers of *Echinacea* with alkylamides or caffeic acid derivatives. *E. purpurea* 95:5 ethanol/water extract inhibited phytohemagglutinin in-dependent production of IL-2, and this inhibitory activity correlated with the presence of alkylamides but not caffeic acid derivatives [9].

A mixture of *E. purpurea* and *Glycyrrhiza glabra* root (Revitonil® tablets) showed a stimulatory effect on human granulocytes, and notable stimulating activity was observed in the T-lymphocyte CD69 bioassay [68]. It found that decreased number and function of regulatory T cells (Tregs) in association with the enhanced feeder function of antigen-presenting cells (APCs) may contribute to the improvement of immune function by *E. purpurea*.

The CD4+ FoxP3+ and CD4+CD25+ Tregs incidence were attenuated, and CD4+CD25+ Tregs function was decreased, while the feeder function of APCs was enhanced in the mice spleens administered with *E. purpurea* for 3 weeks [69].

Echinacea has the potential for enhancement of humoral immune responses as well as innate immunity. An alcohol extract of *Echinacea* improved the T cell-dependent antibody response to immunization with sRBCs in mice and increased splenic T cell production of IL-4 and IL-10

suggesting that *Echinacea* stimulation of both Th-2 and Th-1 cytokine production improved B cell function and increased antibody formation [44]. A diet supplemented with 1% w/w *Echinacea* for 28 days can enhance immune function by increasing antibody production which resulted from augmenting both Th1 and Th2 cytokine production. Production amount of IgA, IgG, and IgM was higher in the splenic lymphocytes of rats fed with *Echinacea* [70].

Arabinogalactan-proteins (AGPs) purified from roots of *E. pallida*, and suspension culture of *E. purpurea* induced the IgM-production of mouse lymphocytes and stimulated IL6-production in alveolar mouse macrophage culture [71]. A study by Rehman and colleagues [6] demonstrated that oral administration *E. angustifolia* root extract of rats for 6 weeks which were injected with the antigen keyhole limpet hemocyanin (KLH) and re-exposed to KLH after the initial exposure, showed a significant augmentation of their primary and secondary IgG response to the antigen [6]. Extract of *E. purpurea* enhanced cellular immune function of PBMC both from normal individuals and patients with either the chronic fatigue syndrome or the acquired immunodeficiency syndrome (AIDS). The addition of herb significantly increased both antibody-dependent and NK-mediated activities against cells infected with herpesvirus [72].

Polysaccharides isolated from *E. purpurea* activated peritoneal macrophages after administration of cyclophosphamide or cyclosporine A (CsA) in immunodeficient mice. *E. purpurea*-treated macrophages exhibited increased production of TNF-α and higher cytotoxicity against tumor target WEHI-164 as well as against the intracellular *Leishmania enrietti*. After a cyclophosphamide-mediated reduction of leukocytes in the peripheral blood, the polysaccharides induced an earlier influx of neutrophil granulocytes.

E. purpurea treatment after cyclophosphamide or CsA restored their resistance against lethal infections with the macrophage-dependent pathogen *Listeria monocytogenes* and predominantly granulocyte-dependent *Candida albicans* [73]. Polinacea® administered orally showed an immune-stimulating activity by reducing the *C. albicans*-induced mortality both in normal and in CsA-treated mice. It enhanced T-cell function and proliferation by stimulating IFN-γ production in anti-CD3-treated murine T-cell cultures [74].

Echinacea-derived alkamides have a structural similarity to anandamide, an endogenous ligand of cannabinoid receptors; consequently, it was found that alkamides bind to cannabinoid receptor (CB) type-1 and -2, therefore suggested as a new class of cannabinomimetics [75]. The CB2 receptor is abundantly expressed in some of the inflammatory and immune cells, and there is evidence that the CB2 receptor plays a role in inflammatory also immune responses, as well as related pathophysiological conditions [76].

N-alkylamides from *E. purpurea* and *E. angustifolia* preparations are capable of stimulating IL-10 and inhibiting expression of TNF-α protein. N-alkylamides exerted pleiotropic effects modulating the endocannabinoid system by activating the CB2 receptor, endocannabinoid transport and degradation [77]. Alkylamides dodeca-2E, 4E, 8Z, 10E/Z-tetraenoic acid isobutylamides (1/2), trienoic (3) and dienoic acid (4) derivatives induced synthesis of TNF-α mRNA in primary human monocytes/macrophages. This up-regulation was found to be mediated by CB2 receptors, increased cAMP, p38/MAPK and JNK signaling, as well as NF-κB and ATF-2/CREB-1 activation [78]. Also *E. purpurea* stimulated prolactin secretion in rats [79,] and it has been described that prolactin plays a significant role in immune system regulation.

A controversy result showed that cynarin isolated from *E. purpurea* down-regulated immune responses by binding to CD28, a receptor of T-cells on T-lymphocytes, and this interaction is stronger than the binding between CD28 and CD80, a co-stimulated receptor of antigen presenting cells. Cynarin's function was proved by its ability to down-regulate CD28-dependent IL-2 expression in a T-cell culture line [80]. It is believed that the immunomodulatory effects of *Echinacea* depend on the combined action of alkamides, polysaccharides, and chicoric acid.

These components are potentially effective in stimulating humoral immunity as well as innate immune responses. It appears likely that *Echinacea*

stimulates the immune system via multiple pathways. *Echinacea* can affect non-specific immune response, activate innate immune cells such as macrophages, polymorphonuclear leukocytes, and NK cells, stimulate phagocytosis, and inhibit the production of inflammatory mediators by white blood cells.

The results about *Echinacea* immunomodulation seem inconclusive and controversial; some studies express that *Echinacea* is an immunostimulatory and some of them state it is an immune suppressor. It is noteworthy to mention that immunity is a two-edged blade that the body keeps under tight control; excessively strong immune reactions can be precarious. Based on this concern, *Echinacea* should be used with caution by individuals with autoimmune disorders such as rheumatoid arthritis and multiple sclerosis. On the other side, *Echinacea* can stimulate immunity and may be harmful where immunosuppression could be vital in some people such as in organ transplantation. Further studies are needed to clarify the mechanisms which are responsible for the beneficial effect of *Echinacea* observed in the immune system.

Anti-Cancer Effects: Interest in herbal medicine for cancer therapy has dramatically grown over the past years. Cancer patients may wish to use natural botanicals to inhibit tumor growth and development. *Echinacea* and its compounds have shown promising anti-tumor effects. The hexanic root extract of the *E. purpurea*, *E. angustifolia* and *E. pallida* reduced cell viability in human pancreatic MIA PaCa-2 and colonic COLO320 cancer cell lines in a concentration- and time-dependent manner. *E. pallida* extract induced apoptosis by increasing caspase-3 activity and the internucleosomal degradation of DNA, without evidence of necrotic cell death [81]. Five compounds, two polyacetylenes (namely, 8-hydroxy-pentadeca-(9E)-ene-11,13-diyn-2-one and pentadeca-(9E)-ene- 11,13-diyne-2,8-dione) and three polyenes (namely, 8-hydroxy-pentadeca-(9E,13Z)-dien-11-yn-2-one, pentadeca- (9E,13Z)-dien-11-yne-2,8-dione and pentadeca-(8Z,13Z)-dien-11-yn-2-one) isolated from the n-hexane extract of *E. pallida* roots induced apoptosis in pancreatic MIA PaCa-2 and colonic COLO320 cancer cells [82].

Ethanolic extract of *E. purpurea* flowers and its major compound cichoric acid showed a significant inhibitory effect on the proliferation of human colon cancer cells Caco-2 and HCT-116 in a dose- and time-dependent manner. Cichoric acid was able to decrease telomerase activity in HCT-116 cells. Moreover, cichoric acid effectively induced apoptosis in colon cancer cells, which were characterized by DNA fragmentation, activation of caspase-9, cleavage of poly-ADP-ribose polymerase (PARP) and down-regulation of β-catenin [19].

An acetylenic constituent of *E. pallida* roots, namely pentadeca-(8 Z,13 Z)-dien-11-yn-2-one, revealed a concentration-dependent cytotoxicity on leukemia Jurkat and HL-60, breast carcinoma MCF-7, and melanoma MeWo cell lines. This component arrested the cell cycle in the G1 phase on HL-60 cells [20]. When a commercially extract of *E. purpurea* root was administered to mice immunized with killed leukemia cells in an erythroleukemic mouse model for 3 months the number of NK cells was elevated compared to animals without receiving the *Echinacea*.

In the group fed with *Echinacea* product in the early phase of the tumor development the number of T- and B lymphocytes in the spleen was markedly enhanced [21]. *E. purpurea* had suppressive effects on spontaneously occurring leukemia caused by endogenous recombinant murine leukemia viruses (MuLV).

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Gastrointestinal mucositis is a common complication of chemotherapeutic drugs, and there is currently no effective long-term treatment. SAMITAL® is an oral suspension formulation based on the combination of three standardized extracts from *Vaccinium myrtillus, Macleaya cordata* fruits and *E. angustifolia* roots designed for the relief of oral mucositis induced by chemotherapy and/or radiotherapy in cancer patients. 20 pediatric patients undergoing chemotherapy initially received oral SAMITAL® to treat gastrointestinal mucositis and were then given SAMITAL® prophylactically to prevent recurrences with successive cycles of chemotherapy. SAMITAL® significantly decreased gastrointestinal mucositis grade, reduced pain, mucosal erosions, bleeding and dysphagia [85]. Phase II trials with SAMITAL® as part of an overall clinical development program are currently ongoing [86].

These results represent scientific evidence on the possible role of *Echinacea* species in medical oncology. Because the agents used for cancer chemotherapy are known to be highly toxic towards normal cells, immune-enhancing activities by *Echinacea* is looked into with interest and more research is needed in this area.

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E. pallida and *E. purpurea* inhibited NO production and TNF-α release from LPS-stimulated RAW 264.7 macrophage cells in response to infection of *Salmonella enterica*. Upon bacterial infection, RAW 264.7 cells produced high levels of NO; however, an alcoholic extract of *Echinacea* administered orally for one week decreased NO production in a dose-dependent manner [87].

The use of *E. purpurea* extract had a prophylactic effect on the development of *Pseudomonas aeruginosa* infection. ***E. purpurea*** feeding resulted in diminishing bacterial number in livers of C57Bl/6 (susceptible strain) and B6C3F1 (relative resistant strain) mice. *Echinacea* feeding of the second relative resistant strain (BALB/cx C3H) F1 resulted in stimulation of granulocytes chemiluminescent and lymphocytes proliferative response [88].

Echinacea could inactivate certain respiratory bacteria and could also reverse the inflammatory effects caused by these bacteria in epithelial cells. Echinaforce® inactivated *Streptococcus pyogenes* (Group A streptococcus), which is often associated with sore throat and more severe pulmonary infections. *Hemophilus influenza* and *Legionella pneumophila* were also readily inactivated, and their cellular pro-inflammatory response completely reversed.

Moreover, *Staphylococcus aureus* (methicillin-resistant and sensitive strains) and *Mycobacterium smegmatis* were also sensitive to Echinaforce®[24] Influenza infection is an important clinical problem. *Echinacea* extracts and active components have the potential for being used in improving the pathology of influenza infections. Echinaforce® has potent antiviral activity against the IV strains, human Victoria (H3N2) and PR8 (H1N1), avian strains KAN-1 (H5N1) and FPV (H7N7), and the pandemic S-OIV (H1N1). Human H1N1-type IV, highly pathogenic avian IV (HPAIV) of the H5 and H7 types, as well as swine-origin IV (H1N1), were inactivated in treatment by Echinaforce®. Hemagglutination assays showed that Echinaforce® inhibited the receptor binding activity of the virus, suggesting that the extract restricted the viral entry into cells.

In sequential passage studies under *in-vitro* treatment with the H5N1 virus, no Echinaforce®-resistant variants appeared, in contrast to Tamiflu, which produced resistant viruses upon passaging. Also, the Tamiflu-resistant viruses were susceptible to Echinaforce® as well as the wild type viruses [26].

The alkyl amides undeca-2Z,4E-diene-8,10-diynic acid isobutylamide, dodeca-2E,4E,8Z, 10E/Z tetraenoic acid isobutylamide, dodeca-2E,4E-dienoic acid isobutylamide, and undeca-2E-ene-8,10-diynoic acid isobutylamide suppressed production of TNF-α and PGE2 from RAW 264.7 macrophage cells-infected with H1N1 influenza. A strain PR/8/34. Dodeca-2E, 4E-dienoic acid isobutylamide especially inhibited production of these mediators and also strongly suppressed

production of granulocyte-colony stimulating factor (G-CSF), MCP-1, MIP-1α and CCL5 (RANTES) [89]. In the context of influenza virus-stimulated human PBMCs from older individuals vaccinated against influenza, *E. tennesseensis* root tinctures augmented IL-10 production, diminished IL-2 production, and had no effect on IFN-γ production [90].

Results indicated that rhinovirus infection of epithelial cells treated with *Echinacea* led to profound effects on numerous mediators. An alcohol tincture from *E. purpurea* roots increased the expression of several transcription factors in a non-activated human bronchial epithelial BEAS-2B cell line but inhibited the expression of these when the cells were infected with rhinovirus. The BEAS-2B cells were used as the model, and nuclear extracts of uninfected cells and rhinovirus-14 infected cells were examined with and without treatment with *E. purpurea* extract. It was found that *Echinacea* increased the nuclear content of several transcription factors, including pro-inflammatory factors such as activator protein (AP)-1, AP-2, NF-κB, and STATs 1-6. Infection by rhinovirus resulted in a more dramatic increase in these transcription factors; however, when rhinovirus-infected cells were treated with *Echinacea*, transcription factor levels were reduced to low levels [39]. Preparations include juice from the aerial parts of the plant (which contain polysaccharides) and alcoholic tinctures from roots (containing caffeic acid derivatives and alkylamides) stimulated the release of pro-inflammatory factors from uninfected BEAS-2B bronchial epithelial cells. Exposure to Rhinovirus 14 stimulated the release of several chemokines known to attract inflammatory cells, and most these effects were reversed by introduction either of the two *Echinacea* extracts [91]. These results could explain the anti-inflammatory effects of *Echinacea*.

The rhinovirus type 1A (RV1A) resulted in increased mucopolysaccharide inclusions in the goblet cells of normal human airway epithelial cells; this change was reversed by *Echinacea* treatment. Echinacea could ameliorate mucus production during colds because mucin secretion stimulated by RV1A is reversed by *Echinacea* treatment. *Echinacea* also inhibited secretion of substantial amounts of the pro-inflammatory cytokines IL-6 and IL-8 in RV-infected tissues [92].

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In an open-label, fixed-sequence study, 50 patients infected by human immunodeficiency virus (HIV) received anti-retroviral therapy including 400 mg etravirine (a non-nucleoside reverse transcriptase inhibitor of HIV) once daily for 4 weeks. Capsules containing *E. purpurea* root extract were added to the anti-retroviral treatment (500 mg every 8 h) for two weeks.

Results showed that the coadministration of *E. purpurea* with etravirine was safe and well tolerated in HIV-infected patients [96]. Cichoric acid has been shown to inhibit the HIV-type 1 integrase, improved the anti-HIV-1 effect of Zidovudine and protease inhibitor (AG1350) *in-vitro* [97].

Echinaforce® inhibited the growth of three species of trypanosomatids: *Leishmania donovani*, *Leishmania major*, and *Trypanosoma brucei*. *L. donovani* stimulated the production of the pro-inflammatory IL-6 and IL-8 cytokines in human bronchial epithelial cells and skin fibroblasts, but Echinaforce® eradicated this stimulation by anti-inflammatory effect [98]. By application of purified polysaccharides from cell cultures of *E. purpurea*, peritoneal macrophages killed cells infected either with the parasite *Leishmania enriettii*, or with yeast *Candida albicans* [99].

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These results provide evidence that bacterial, viral, and fungal infections could be halted by *Echinacea* administration.

Anti-Cancer Effects: Interest in herbal medicine for cancer therapy has dramatically grown over the past years. Cancer patients may wish to use natural botanicals to inhibit tumor growth and development. *Echinacea* and its compounds have shown promising anti-tumor effects.

The hexanic root extract of the *E. purpurea*, *E. angustifolia*, and *E. pallida* reduced cell viability in human pancreatic MIA PaCa-2 and colonic COLO320 cancer cell lines in a concentration- and time-dependent manner. *E. pallida* extract induced apoptosis by increasing caspase-3 activity and the internucleosomal degradation of DNA, without evidence of necrotic cell death [81].

Five compounds, two polyacetylenes (namely, 8-hydroxy-pentadeca-(9E)-ene-11,13-diyn-2-one and pentadeca-(9E)-ene- 11,13-diyne-2,8-dione) and three polyenes (namely, 8-hydroxy-pentadeca-(9E,13Z)-dien-11-yn-2-one, pentadeca- (9E,13Z)-dien-11-yne-2,8-dione and pentadeca-(8Z,13Z)-dien-11-yn-2-one) isolated from the n-hexane extract of *E. pallida* roots induced apoptosis in pancreatic MIA PaCa-2 and colonic COLO320 cancer cells [82].

Ethanolic extract of *E. purpurea* flowers and its major compound cichoric acid showed a significant inhibitory effect on the proliferation of human colon cancer cells Caco-2 and HCT-116 in a dose- and time-dependent manner. Cichoric acid was able to decrease telomerase activity in HCT-116 cells. Moreover, cichoric acid effectively induced apoptosis in colon cancer cells, which were characterized by DNA fragmentation, activation of caspase-9, cleavage of poly-ADP-ribose polymerase (PARP) and down-regulation of β-catenin [19].

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The Common Cold and Upper Respiratory Infections: The common cold is one of the most common illnesses worldwide, coupled with a lack of specific treatment options which invites to evaluate alternative therapies such as herbal remedies. Nowadays, *Echinacea* is possibly the most recognized herbal supplement for the prevention and treatment of colds.

Many people believe that *Echinacea* can boost the immune system and reduce the severity and the frequency of cold symptoms. This plant is widely used to fight the common cold and other upper respiratory infections in the United States and Europe, nevertheless, some studies of *Echinacea* for the common cold have not found that it really helps. The clinical effectiveness of *Echinacea* is controversial because clinical trials have had mixed results; some studies have shown clinical benefit, whereas others have not (summarized in **Table 2**).

Some studies showed that *Echinacea* could reduce duration, severity, and frequency of symptoms of cold and URIs. Hoheisel *et al*., in a double-blind study found that *E. purpurea* pressed juice reduced the duration and the severity of colds. In this study, 120 people were given *E. purpurea* or a placebo as soon as they started showing signs of getting a cold. Treatment with *Echinacea* at the early onset of cold or flu symptoms was effective for relieving these symptoms in a shorter period [11].

The results from a clinical study suggested that treatment with Echinaforce® can be recommended as a prophylactic treatment. Long-term treatment with Echinaforce® was associated with a significant reduction in both total number and severity of cold episodes, whereas it did not induce any health risk above that reported with the placebo treatment [101].

In a double-blind trial, 246 healthy adult volunteers with recent onset of respiratory infection were given either Echinaforce®, *E. purpurea* concentrate (same preparation at 7 times higher concentration), special *E. purpurea* radix preparation or placebo until they felt healthy again but not longer than 7 days. Echinaforce® and its concentrated preparation were significantly more effective than the special *Echinacea* extract or placebo.

All treatments were well tolerated, and among the *Echinacea* groups, the frequency of adverse events was not significantly higher than in the placebo group [102].

In a randomized, double-blind, placebo-controlled trial, 282 subjects with a history of two or more colds in the previous year, but otherwise in good health, were recruited. They were instructed to start Echinilin (a formulation prepared from freshly harvested *E. purpurea* and standardized by active components alkamides, cichoric acid, and polysaccharides) or placebo at the onset of the first symptom related to cold for seven days.

The total daily symptom scores were found lower in the Echinilin group, and the response rate to treatments was greater in the treated group. Early intervention with a standardized formulation of *Echinacea* resulted in reduced symptom severity in subjects with naturally acquired upper respiratory tract infection [103].

In a study, Echinilin was administered to 150 adults at the onset of their cold for one week, with eight doses (5 ml/dose) on day 1 and three doses on the subsequent days. Researchers observed it decreased daily symptomatic scores and sustained increase in the number of circulating total white blood cells, monocytes, neutrophils, and NK cells in the *Echinacea* group versus the placebo group. These results suggest that Echinilin, by enhancing the non-specific immune response and stimulating free radical scavenging properties, may have led to a faster resolution of the cold symptoms [104].

In one double-blind placebo-controlled study, a total of 95 subjects with early symptoms of cold or flu (runny nose, scratchy throat, fever) were randomly assigned to receive an *Echinacea* herbal tea preparation (Echinacea Plus) 5-6 cups/day titrating to 1 over 5 days or placebo, the study period was 3 months. There was a significant difference between the *Echinacea* and placebo group, also there were no negative effects reported by any of the subjects in either group [10]. 473 patients with early influenza symptoms randomized to either 5 days of oseltamivir followed by 5 days of placebo, or 10 days of an *E. purpurea*-based formulation called Echinaforce Hot drink (Switzerland).

According to the results, Echinaforce Hot drink is as effective as oseltamivir in the early treatment of influenza infections with a reduced risk of complications and adverse events [105]. In a prospective, double-blind, randomized study, 62 patients with symptoms of the common cold were treated with a natural multiherbal formula Immumax (containing *Echinacea* extract, garlic powder, *Nigella sativa* oil, and *Panax ginseng* extract plus vitamin C and zinc) for the duration of their symptoms or a maximum of 2 weeks.

The results indicated that Immumax helped reduce the duration and severity of common cold symptoms, and the symptoms resolved faster in the Immumax group than in the placebo group. The median time to resolution of all symptoms was 8 days in the placebo group and 4 days in the Immumax group [106]. An herbal compound of *E. angustifolia*, Arabinogalactan, Vitamin C, Beta-Glucan e Zinc (Imoviral® Junior) was given to 37 children affected by recurrent pharyngotonsillitis or otitis media. Almost all children after six months reported a reduction in the frequency of acute episodes.

Imoviral® Junior can improve the quality of life in pediatric patients affected by recurrent pharyngotonsillitis and otitis media without adverse effects [107]. URIs frequently cause exacerbations of chronic obstructive pulmonary disease (COPD). In a double-blind, randomized placebo-controlled trial in COPD patients with acute URI, the combination of *E. purpurea* along with micronutrients zinc, selenium and vitamin C may alleviate COPD exacerbations caused by acute URI [108].

In contrast to abovementioned results, some studies have reported no statistically significant improvement with *Echinacea* for the common cold. Taylor *et al.*, [109] using a large randomized controlled trial found no evidence of *E. purpurea* help in children suffering from URIs. Results of this trial do not support a benefit of *Echinacea* in the treatment of common cold symptoms in children, and its use was associated with an increased risk of rash. However, the authors concluded that their findings are not transferable to *Echinacea* use in adults or other species/preparations of *Echinacea*. In another randomized, double-blind placebo-controlled design, 128 adults received either 100 mg of *E. purpurea* (freeze-dried pressed juice from the aerial parts) or placebo 3 times daily until cold symptoms were relieved up to a maximum of two weeks.

The time to resolution of symptoms was not statistically different, and no significant difference was observed between treatment groups for symptoms including sneezing, nasal discharge, nasal congestion, headache, sore or scratchy throat, hoarseness, muscle pain, and cough [110]. A total of 109 patients with a history of more than three colds or respiratory infections in the previous year were randomly assigned to receive 4 ml *E. purpurea* fluid extract twice a day in a double-blind study.

Treatment with *E. purpurea* did not significantly decrease the incidence, duration or severity of colds and respiratory infections compared to placebo. Adverse events were observed in 20% of patients in the *Echinacea* group compared with 13% of patients in the placebo group [111].

In a randomized, double-blind clinical trial 58 subjects were assigned to survey the effects of *Echinacea* on the frequency of upper respiratory symptoms. Individuals in the *Echinacea* group reported 9 sick days during 8 weeks, whereas the placebo group reported 14 sick days. No difference was found in the frequency of upper respiratory tract symptoms and total symptom days for patients taking prophylactic *Echinacea* for the 8-week period compared with those taking parsley capsules.

The findings suggest that *Echinacea* does not have a meaningful effect on respiratory tract infection symptoms.[112] In a large randomized study on 713 patients, Barrett *et al*. found that dried *E. purpurea* and *E. angustifolia* root (10.2 g for the first 24 h of a cold and 5.1 g for the next 4 days) did not improve symptoms more than placebo or no treatment. Change in IL-8 levels and neutrophil counts were also not statistically significant in the no-pill group [113].

Barret *et al*. in another randomized, double-blind placebo-controlled study on adults with colds concluded *E. angustifolia* and *E. purpurea* does not have an expressive effect [114]. In a double-blind trial by Melchart *et al.,* 302 healthy volunteers were given an alcohol tincture containing either *E. purpurea* root, *E. angustifolia* root, or placebo for 12 weeks. The results showed that *E. purpurea* and *E. angustifolia* decreased the number of people who got sick; however, the difference was not statistically significant [115]. Three meta-analyses evaluated the effect of *Echinacea* on incidence, duration, and prevention of the common cold and induced rhinovirus infections [12-14]. Schoop *et al*., performed a meta-analysis of experimental rhinovirus infection studies with humans to determine whether the negative results obtained in previous studies were a consequence of efficacy or inadequate sample size. A total of 234 articles were identified through the literature search; and based on the analysis, the likelihood of experiencing a clinical cold was 55% higher with placebo than with *Echinacea*. This meta-analysis exhibited standardized extracts of *Echinacea* were effective in the prevention of symptoms of the common cold after clinical inoculation, compared with placebo [12]. Shah *et al*. did meta-analysis reviewed *Echinacea* clinical trials that examined both prevention and treatment in the incidence and duration of the common cold.

The results supported *Echinacea*'s benefit in decreasing the incidence and duration of the common cold.[13] Evidence from another meta-analysis with a total of 2458 participants by Schapowal *et al*. indicated that the use of *Echinacea* potently reduced the risk of recurrent respiratory infections and the development of complications. Ethanolic extracts from *Echinacea* appeared to be more effective than pressed juices and increased dosing during acute episodes further enhanced these effects [14]. A Cochrane review by Karsch-Völk *et al.* [116] Compared the effect of *Echinacea* to placebo, in the treatment and the prevention of the common cold. They reviewed twenty-four double-blind controlled clinical trials with 4631 participants investigating the effectiveness of several different *Echinacea* preparations for preventing and treating common colds or induced rhinovirus infections. The authors concluded that *Echinacea* products have not proved statistically significant in reducing illness occurrence, although there might be a weak benefit from some *Echinacea* products.

TABLE 2: CLINICAL TRIALS AND HUMAN STUDIES ON EFFICACY OF *ECHINACEA* AGAINST COMMON COLD AND UPPER RESPIRATORY TRACT INFECTIONS

Intervention	Study population	Type of study	Comparator	Dosage/Treatment period	Outcome	References
Tea preparation from aerial parts of *E. purpurea* and *E. angustifolia*, and *E. purpurea* root	patients with the earliest symptoms of a cold	RCT	Placebo	5 to 6 cups day 1, titration to one cup on day 5/ 5 days	Positive	[10]
Pressed juice of *E. purpurea* herb (Echinacin®)	patients with symptoms of a cold	RCT	Placebo	20 drops every 2 h on day 1 followed by 3 × 20 drops/day/ 10 days	Positive	[11]
Echinaforce® (alcoholic	healthy adults	RCT	Placebo	for illness prevention: 3	Positive	[101]

extraction from freshly harvested *E. purpurea* with 95% herb and 5% roots)				× 0.9 ml/day; in case of cold: 5 × 0.9ml/day/ 4 months		
Echinaforce® tablets (*E. purpurea* crude extract based on 95% herb/5% roots)	healthy volunteers	RCT	Placebo	2 × 3 (40.68 mg/day)/ maximum one week	Positive	[102]
Freshly harvested *E. purpurea* and standardized by active components alkamides, cichoric acid and polysaccharides (Echinilin®)	healthy adults	RCT	Placebo	10 × 4 ml the first day, then 4 × 4 ml/ 7 days	Positive	[103]
E. purpurea (Echinilin®)	adults at the onset of their cold	RCT	Placebo	8 × 5 ml for day 1, then 3 × 5 ml for 6 days/ one week	Positive	[104]
Syrup of *E. purpurea* herb harvested at flowering	healthy children	RCT	Placebo	3.75 ml twice a day for ages 2-5 years and 5 ml twice a day for ages 6-11 years/ 10 days	Negative	[109]
Echinacea fresh capsule (freeze-dried pressed juice from *E. purpurea*)	patients with cold	RCT	Placebo	3 × 1 capsule/day/ maximum 14 days	Negative	[110]
Pressed juice of *E. purpurea* (Echinacin®)	patients with symptoms of a cold	RCT	Placebo	2 × 4 ml daily/ 8 weeks	Negative	[111]
Capsules of *E. purpurea* dried plant	healthy adults	RCT	Placebo	3 × 2 capsules/day (1 capsule containing 300 mg *E. purpurea*)/ 8 weeks	Negative	[112]
Tablets containing *E. purpurea* root and *E. angustifolia* root	individuals with cold symptoms	RCT	Placebo	4 × 2 tablets during the first day, then 4 × 1 tablet per day for the next 4 days/ 5 days	Negative	[113]
Capsules containing 50% *E. angustifolia* root, 25% *E. purpurea* root, 25% *E. purpurea* herb	patients with active cold	RCT	Placebo	6 × 4 capsules on the first day, then 3 × 4 capsules up to 10 days/ 10 days	Negative	[114]
E. Purpurea/ E. angustifolia root alcoholic extract	healthy volunteers	RCT	Placebo	50 drops twice a day/ 12 weeks	Negative	[115]

RCT = Double-blind, randomized, controlled trial

Studies of *Echinacea* for the common cold have had mixed results, and whether or not *Echinacea* helps prevent or treat the common cold remains under debate. There are many variables in studying *Echinacea* for the common cold and URIs.

All studies to date have had essential limitations, including differences in trial design, use of different species and plant parts (containing different constituents), various routes of administration, sample size, monitoring responses of healthy subjects, analytical methodology, and choice of biomarker and placebo. These differences make it hard to compare the results; consequently, these data do not allow clear conclusions whether *Echinacea* might be effective for this purpose or not.

Hepatoprotective Effects: Chicoric acid may reduce acute alcohol-induced steatosis in mice through interfering with the induction of iNOS and iNOS-dependent signaling pathways in the liver. Acute alcohol administration caused a significant increase in hepatic triacylglycerols accumulation, which was associated with increase in 4-hydroxynonenal protein adducts, and iNOS and

active plasminogen activator inhibitor 1 protein level in the liver; pretreatment with chicoric acid for 4 days before ethanol ingestion significantly attenuated these alcohol effects on the liver.

In RAW264.7 macrophages, treatment with chicoric acid (4 mg/kg body weight) suppressed LPS-induced TNF-α and iNOS at mRNA expression level [23]. *E. purpurea* extract had a protective role on kidney and liver against diethylnitrosamine (DEN) toxicity in rats. One month administration of DEN caused an elevation in serum alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), creatinin and total and direct bilirubin levels in serum; however these factors decreased in *E. purpurea* administration. Also, the histopatho-logical investigation revealed a proliferation of hepatic stellate cells [117]. It was found that alkamide dodeca-2E, 4E, 8Z, 10Z(E)-tetraenoic acid isobutylamides isolated from *E. purpurea* root has hepatoprotective effect against acute fulminant hepatitis induced by lipopolysaccharide/D-galactosamine (LPS/D-GalN) in mice.

This dose-dependently induced HO-1 protein expression in LPS-stimulated murine macrophages that were regulated through an increase in JNK phosphorylation, c-jun protein expression, and phosphorylation, and transcription factor AP-1 binding consensus DNA activity. Besides, this alkamide suppressed serum aminotransferase activities, TNF-α expression, and damages to hepatocytes of LPS/d-GalN-treated mice [118]. Cadmium (Cd^{2+}) is toxic to a wide range of tissues. Prolonged intraperitoneal injection of *E. purpurea* extract combined with Cd(2+)Cl(2) decreased the mitotic activity induced by Cd^{2+}, also increased apoptotic activity of hepatocytes of mice [119]. These data suggest a new application of *Echinacea* as a hepatoprotective agent; however further investigation is needed.

Echinacea against Diabetes: Diabetes is a debilitating and often life-threatening problem with increasing incidence throughout the world. A scientific investigation of herbal remedies for diabetes may provide valuable alternative drugs and therapeutic strategies. Recent studies have indicated that *Echinacea* could have anti-diabetic activities.

Adipogenesis has been used to study the insulin signaling pathway and to screen anti-diabetic compounds. When adipocyte differentiation was induced with insulin plus 3-isobutyl-1-methylxanthine and dexamethasone, the accumulation of lipid droplets and the cellular triglyceride content were increased by ethanolic extract of *E. purpurea*. The expression of PPAR-γ and CCAAT-enhancer-binding protein (C-EBP)α in 3T3-L1 adipocytes-treated with dodeca-2(E),4(E)-dienoic acid isobutylamide were increased, as well as triglyceride content of adipocytes and fat accumulation, were improved [28]. The isomeric C12-alkamides isolated from a dichloromethane root extract of *E. purpurea* were found to activate PPAR-γ, to increase basal and insulin-dependent glucose uptake in 3T3-L1 adipocytes in a dose-dependent manner, and to exhibit characteristics of a PPAR-γ partial agonist [29].

The alkamide hexadeca-2E,9Z,12Z,14E-tetraenoic acid isobutylamide isolated from an n-hexane extract of *E. purpurea* flowers was shown to increase insulin-stimulated glucose uptake and to activate PPAR-γ without stimulating adipocyte differentiation [30].

In non-obese diabetic (NOD) mice, supplementation with *Echinacea* resulted in a significant increase in the absolute numbers of NK cells in the spleen, irrespective of feeding duration; moreover, it stimulated NK cell production in their bone marrow origin site. What's more, consumption of herb by NOD mice has led to no negative consequences concerning the hemopoietic and immune lineages [120]. These data suggest that *Echinacea* contain compounds with the potential to manage insulin resistance and diabetes.

Effects on Skin Problems: Several plants with effective properties may offer better alternative treatments for skin complications. *Echinacea* contains many valuable constituents for protection of skin from oxidative stress and for improving skin health. In a survey, *E. purpurea* extract was incorporated into cream and gel bases, and the effect of those formulations on skin irritation, hydration level, and wrinkle reduction was evaluated in 10 healthy volunteers.

According to results *E. purpurea* cream and gel were effective in improving skin hydration and reducing wrinkle. Also both formulations showed no irritation to skin [121]. Acne is a chronic inflammatory disorder of skin follicles caused by the gram-positive bacterium *Propionibacterium acnes*. Echinaforce® might provide a useful, safe treatment in the control of acne disease by preventing the proliferation of the organism and inhibiting the bacterial-induced inflammation. In human skin fibroblasts *in-vitro*, *P. acne* induced the secretion of considerable amounts of pro-inflammatory cytokines IL-6 and IL-8; however, Echinaforce® completely reversed their level to normal [22]. *Echinacea* is purported to be beneficial for wound healing. It revealed that root extract of *E. pallida* and its constituent echinacoside has good wound healing properties in excision wounds treated topically. This activity probably related to the anti-hyaluronidase and anti-inflammatory activities of echinacoside [122].

Alcoholic extract of *E. pallida* accelerated cutaneous wound closure in the stressed mice but had no curing effect for the non-stressed mice. The wound healing effect of *Echinacea* in stressed mice is not mediated through modulation of glucocorticoid signaling because plasma glucocorticoid in restraint-stressed mice treated with *Echinacea* didn't change [123].

Effects on Nervous System: Excitatory synaptic transmission in the hippocampus, a brain region that is involved in anxiety and anxiety-related behaviors, was suppressed by an *Echinacea* extract; but no change in inhibitory synaptic transmission could be detected upon application of this extract.

Also, at low concentration the *Echinacea* extract reduced the spiking activity of CA1 pyramidal cells, while at high concentration increased it, this was parallel to the reduction in the magnitude of the h-current-mediated voltage responses in pyramidal cells [124].

Anxiolytic potency of *Echinacea* preparations was consistently seen in three different laboratory tests of anxiety, the elevated plus-maze, social interaction, and shock-induced social avoidance tests; these effects are comparable with chlordiazepoxide. *Echinacea* preparations had considerable anxiolytic potential, and it decreased anxiety-like behavior in all tests [125]. *E. angustifolia* extract decreased anxiety in the elevated plus-maze and ameliorated contextual conditioned fear. No lethality or behavioral signs of discomfort were noticed in rats treated with intragastric administration of 1000 and 3000 mg/kg *E. angustifolia*.

A pharmacological formulation based on the same *E. angustifolia* extract was tested in healthy volunteers. One or two tablets (each containing 20 mg of the plant extract) per day were administered for 7 days to subjects scoring high on the State-Trait Anxiety Inventory (STAI). The high dose (2 tablets/day) decreased STAI scores within 3 days in human subjects, an effect that remained stable for the duration of the one-week treatment and for the 2 weeks that followed treatment [126]. The endocannabinoid system involves many pathophysiological activities including analgesic action.

Echinacea-isolated alkamides appear to exert psychoactive activities through the endocannabinoid system [77].

Alkamides from *E. angustifolia* roots showed psychoactive properties by binding to CB1 receptors predominantly expressed in the brain tissue [127]. The finding of the interaction of alkamides with CB receptors may help explain the traditional use of *Echinacea* for wound healing, pain relief and improvement of cold symptoms [128].

Effects on the Reproductive System: Two studies raised questions about possible antifertility effects of *Echinacea*. The histological changes were found after 4 or 8 weeks of using 50 mg/kg *E. purpurea* in Wistar male rats.

Results of the study showed a significant reduction in the testicle mass and the body mass, as well as changes in histological structures after eight weeks of *E. purpurea* administration. Consumption of the preparation for 8 weeks caused anti-androgenic variations in cells of the spermatogenic epithelium of a testicle duct and consequently inhibited spermatogenesis [129]. An *in vitro* investigation by fresh sperm specimens exhibited *E. purpura* at high-concentration interfered with sperm enzymes, possibly the *Echinacea* treatment may prevent

sperm from fertilizing the oocytes. The beat cross frequencies were higher for *Echinacea* treatment group [130].

Echinacea extract can be a useful medication for the treatment of benign prostate hyperplasia (BPH). Results of work showed a significant decrease of prostate weight of BPH in rats and reversed changes in the structure of the prostate gland after using *E. purpurea* extract for 8 weeks [84]. *E. angustifolia* extract enhanced cell viability and proliferation in mammary epithelial cells, HC11 mouse cell line and BME-UV bovine cell line.

This effect may be modulated by MAPK and Akt activation and by a reduction of caspase-3 activity in *Echinacea* treatment. Moreover, *Echinacea* was able to increase β-casein expression in association with prolactin (5 mg/ml). These data demonstrate that *E. angustifolia* extract can stimulate mammary epithelial cell physiology and may be considered a candidate to support mammary gland activity during mammogenesis and lactogenesis states [131].

Metabolism and Pharmacokinetics: It is found that the application of herbal medicinal products and their components at the same time with substances that are cytochrome P450 (CYP) enzymes substrates may cause herb-drug interactions. Since the use of herbal supplements continues to increase throughout the world, the potential for drug-herbal interactions also rises.

Modified expression of CYP enzymes will affect drug metabolism in three different ways. It can alter drug elimination, pro-drug activation, or drug bioactivation (such as conversion to toxic metabolites), all of which can have serious consequences [132]. *Echinacea* is a widely used herbal medicinal product, and consequently, studies of its interactions with conventional drugs are of particular importance. It was demonstrated *E. purpurea* ethanolic extract could potently inhibit the expression of CYP3A1 and CYP3A2, also CYP2D2 and CYP2C6 activities [133]. CYP3A4 and CYP2C9 enzymes have been reported to metabolize one of the main alkamide constituents of *E. purpurea* extract in human liver microsomes [134].

Administration *E. purpurea* root extract for 8 days to healthy volunteers significantly decreased CYP1A2 activity, increased hepatic CYP3A4 activity, and there was little decrease in CYP2C9 activity. *E. purpurea* root reduced the oral clearance of substrates of CYP1A2 but not the oral clearance of substrates of CYP2C9 and CYP2D6 [135]. *E. purpurea* cause herb-drug interaction by up-regulating CYP1A2, CYP3A4, and MDR1 expression via pregnane X receptor (PXR) activation in human liver carcinoma HepG2 cells [136].

Alkylamide undeca-2 E,4 E/ Z-diene-8,10-diynoic acid isobutylamide correlated well with inhibition of CYP3A4 by Echinaforce® [137]. In a clinical study, no significant effect of *E. purpurea* on midazolam pharmacokinetics was reported in healthy volunteers. *E purpurea* selectively altered the catalytic activity of CYP3A in the liver vs. intestine, conversely, *E. purpurea* whole plant extract administration did not significantly alter CYP3A metabolic serum ratios of 1-hydroxymidazolam: midazolam in healthy volunteers [138].

E. purpurea significantly induced cytochrome CYP3A activity but did not alter lopinavir-ritonavir exposure in healthy subjects. Neither lopinavir nor ritonavir (400/100 mg) pharmacokinetics was significantly altered by 2 weeks of *E. purpurea* coadministration in healthy volunteers. *E. purpurea* induced CYP3A activity but did not alter lopinavir concentrations, most likely due to the presence of ritonavir as a potent CYP3A inhibitor.

E. purpurea is unlikely to alter the pharmacokinetics of ritonavir-boosted protease inhibitors but may cause a decrease in plasma concentrations of other CYP3A substrates [139]. In an open-label, fixed-sequence study the interaction of *E. purpurea* with etravirine, a non-nucleoside reverse transcriptase inhibitor of HIV was investigated. Fifteen HIV-infected patients receiving etravirine (400 mg once daily) for 4 weeks, and *E. purpurea* root extract (500 mg every 8 h) was added to the anti-retroviral treatment for 14 days. Results showed that the coadministration of *E. purpurea* with etravirine was safe and well tolerated in HIV-infected patients [96].

The multidrug transporter P-glycoprotein (P-gp), the product of the ABCB1 gene, involved in cancer multidrug resistance (MDR) and in herb-drug or

drug-drug interactions. Concomitant administration of medicinal herbs with drugs that are P-gp substrates may produce clinically significant herb-drug interactions. P-glycoprotein is responsible for exporting xenotoxins including pharmaceutical medicines from the cell.

Alkamides isolated from *E. angustifolia* inhibited P-gp-mediated calcein transport a major constituent of the blood-brain barrier in isolated porcine brain capillary endothelial cells [140]. The n-hexane root extracts from *E. pallida*, *E. angustifolia*, and *E. purpurea* reduced the efflux of the P-gp probe calcein-AM from human proximal tubule HK-2 cells (that constitutively expresses ABCB1). Pentadeca-(8 Z,13 Z)-dien-11-yn-2-one isolated from the n-hexane extract of *E. pallida* roots was an efficient compound that reduced P-gp activity and decreased the calcein-AM efflux [141]. The interaction of *E. purpurea* with P-gp transporter was studied in human adenocarcinoma colonic cell line Caco-2, as a standard rapid, reliable, and low-cost model for study the absorption of drugs by the intestine.

In a study, digoxin was used as a substrate and verapamil as a control inhibitor. At high concentrations of *E. purpurea*, a significant linear decrease was observed in the net digoxin flux, indicating a dose-dependent inhibitory effect of *E. purpurea* on P-gp [142].

Commonly used herbal supplements were screened for their potential to inhibit UDP-glucuronysl transferase 1A1 (UGT1A1) activity using human liver microsomes. *Echinacea* showed inhibition of UGT1A1 activity [143]. Alkamides and cinnamic acid have been shown to have good permeability through human Caco-2 cells, although caftaric acid, echinacoside, and cichoric acid permeated poorly through the Caco-2 monolayers [144].

These findings suggest that *Echinacea* and components may influence the metabolism of different drugs also alter their pharmacokinetics. However, further studies are also needed to confirm the potential of interactions between *Echinacea* and other conventional drugs.

Safety and Toxicity: The increasing use of medicinal herbs among the populations has created the need for scientific research to determine the safety of herbs. Herbal remedies and dietary supplements are not classified as drugs by the US Food and Drug Administration (FDA); therefore, although the 1994 Dietary Supplement Health and Education Act allows manufacturers to make claims intended to influence public opinion regarding the benefits of these products, herbs and supplements are excepted from the rigorous regulations required for drugs.

Echinacea has an excellent safety record and is well tolerated by most consumers. Oral administration of the *E. purpurea* expressed juice for 4 weeks proved virtually non-toxic to rats and mice, even in doses many times greater than human therapeutic dose [145].

There are reports of anti-apoptotic activity of *E. angustifolia* extract on noncancerous cells. The biological effect of *E. angustifolia* extract on cell viability, and cell differentiation in mammary epithelial cells have been observed in two different cell lines HC11 and BME-UV. This herb activated MAPK/Akt pathway involved in cell viability and proliferation and prevention of caspase-3 accumulation that indicates an anti-apoptotic effect of *Echinacea* extract [131]. Moreover, hydroalcoholic extract and pressed juice from *E. pallida* exhibited a low cytotoxic effect on monkey kidney cell cultures [146].

Cleft palate is one of the most common birth defects. *E. purpurea* extract had a prophylactic effect on the incidence of phenytoin-induced cleft palate. In a study, the prophylactic effects of levamisole and *Echinacea* extract on teratogenic effects of phenytoin were compared.

This study was performed on pregnant mice that received phenytoin at 10th day of gestation, *E. purpurea* extract was administrated at a dose of 360 mg/kg intraperitoneally, along with and 12 hours after phenytoin injection. Cleft palate incidence decreased in fetuses of mice that received phenytoin with *Echinacea* extract; also mean weight and length of fetuses of the ones that received *Echinacea* were significantly greater than those received only phenytoin [147].

The teratogenic effect of phenytoin on the cleft palate is associated with inhibition of cell proliferation and increase in cell apoptosis of

mouse embryonic palatal mesenchymal (MEPM) cells, and *E. purpurea* extract had the reverse effect [148].

On the other side of safety, there are rare cases of adverse effects. Such side effects include nausea, abdominal pain, diarrhea, itch, and rash. *Echinacea* has also been linked to allergic reactions, including asthma, shortness of breath, and one case of anaphylaxis [149]. *Echinacea* can be associated with allergic reactions that may be severe or exacerbate asthma [149]. Moreover, it has also been incriminated in causing contact dermatitis and anaphylactic reactions associated with the consumption of *Echinacea* [150].

In clinical trials, gastrointestinal symptoms were common. *Echinacea* supplementation has altered the gastrointestinal tract microbiota. In study 50 human subjects consumed 1000 mg of standardized *E. purpurea* for 10 days. Significant increases were found for total aerobic bacteria, Bacteroides group, and Bacteroides fragilis after *E. purpurea* exposure [151]. There are concerns that by stimulating immune function, *Echinacea* could potentially exacerbate autoimmune diseases and decrease the effectiveness of immunosuppressive drugs, but this warning is based on theoretical considerations rather than human analyses.

Its immunostimulatory property may lead to interference with immunosuppressant agents; therefore a consensus exists about the avoidance of *Echinacea* in patients who are being administered immunosuppressive drugs, especially in patients undergoing organ transplantation. However, despite the immunostimulatory effect that may be seen with short-term use, chronic long-term use (>6-8 weeks) of *Echinacea* may be immunosuppressive [152] which increases the risks of poor wound healing and opportunistic infections.

Consequently, its use in AIDS or autoimmune disorders such as multiple sclerosis and rheumatoid arthritis is controversial. Furthermore, there are also concerns about its potential hepato-toxicity, probably the use of *Echinacea* in combination with other drugs metabolized by the liver can cause possible unwanted effects; thus this plant should be used carefully in patients with pre-existing hepatic dysfunctions. So altogether, while *Echinacea* administration appears to be without significant adverse effects, the full toxicological profile of *Echinacea* remains to be assessed.

CONCLUSION: *Echinacea* is one of the most important medicinal herbs. In a 2007 National Center for Complementary and Alternative Medicine survey, *Echinacea* was the third most commonly used non-vitamin, non-mineral natural product among adults, and the fifth highest selling herbal dietary supplement [153]. At present, extracts and plant products made from *Echinacea purpurea*, *E. angustifolia*, and *E. pallida* comprise one of the largest parts of the herbal medicine market, in North America as well as in Europe.

Echinacea is widely used as one of the treatments chosen by consumers with the belief that it will reduce the severity and duration of the common cold and because of its supposed beneficial effects on the immune system. There are multiple scientific studies published to evaluate the effects of *Echinacea* on common cold and immunity; however, results are controversial and inconclusive.

It is well known that the effectiveness of distinct *Echinacea* products differs considerably, mainly due to the use of different parts of the plant (including leaves, flowers, root or rhizome), extraction protocols, and the addition of other components. These all have made their outcomes difficult to interpret and compare.

Different commercial products are likely to contain different relative concentrations of active constituents, and there are currently no regulated manufacturing standards for the amount of individual chemical compounds in commercial preparations. Also, the dose and timing of administration also need to be evaluated extensively.

Results show that among the bioactive compounds present in *Echinacea*, alkylamides, cichoric acid and polysaccharides have most effects. However, the biological impacts of unidentified compounds in this herb should be also investigated.

Despite many pharmacological and clinical studies, the molecular mechanisms for *Echinacea* which exert its effects are not well understood. Additionally, more scientific research is required regarding its safety and adverse effects.

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