

Antioxidant Effects of Echinacea Purpurea Nanoparticles for Oral Medicine Applications

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Abstract:

An equally famed medicinal plant, Echinacea purpurea, has been a focal point of studies focusing on its possible health benefits, especially its antioxidant properties. Recent advances in nanotechnology have facilitated the creation of Echinacea purpurea nanoparticles (EPNs), which may improve the bioavailability and the efficacy of its bioactive compounds. This study investigates the antioxidant property of EPNs with a series of in vitro and in vivo assays, observing any cellular mechanism involved therein. EPNs were shown to effectively scavenge free radicals and mouse skin lymphocyte oxidative stress, much more than Echinacea purpurea standard extracts. Further work examined the cytoprotective EPN activities on several cell lines exposed to oxidative injury, an indication to strengthen cellular defense against free radicals. The findings suggest that Echinacea purpurea nanoparticles may be good candidates for natural antioxidants to be further investigated for application in nutraceuticals and therapy against disorders related to oxidative stress. Clinical studies are needed to understand the pharmacokinetics and long-term effects of EPN.

Keywords: Nanoparticles, Echinacea purpurea, Antioxidant, flavonoids, phenolic acids.

Introduction:

Echinacea purpurea, popularly known as the purple coneflower, is a perennial herb native to North America and a member of the family Asteraceae¹. Traditionally, Echinacea has been used by various indigenous cultures for its medicinal properties to treat wounds and respiratory problems. In modern herbal medicine, however, Echinacea has gained a reputation for supporting and stimulating the immune system and combating free radical damage due to the numerous pharmacologically active compounds it contains, including flavonoids, glycoproteins, and derivatives of caffeic acid². These compounds have proven to exhibit strong antioxidant, anti-inflammatory, and immunomodulatory activities, thereby marking Echinacea as a recognised healer for conditions about oxidative stress, including but not limited to cardiovascular disease, cancer, and neurodegenerative processes.

Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defence. This imbalance is an important factor contributing to the pathology of various chronic diseases. Antioxidants act by scavenging ROS, thereby preventing cellular damage and inflammation⁽³⁾. Echinacea purpurea extracts exhibited excellent free radical-scavenging properties due to the presence of high concentrations of cichoric acid and other phenolic compounds. Nevertheless, traditional herbal extracts often have to contend with problems of variable potency, poor bioavailability, and instability in storage or upon processing. Hence, innovative approaches must be introduced to counter these limitations and improve the therapeutic efficacy of Echinacea's active ingredients⁽⁴⁾.

The innovation of nanotechnology has revolutionised biomedicine by providing avenues for the manipulation of substances on a nanometric scale to impart solubility, stability, and bioavailability. Antioxidant therapy shows promise to be furthered by the introduction of nanoparticles derived from the phytochemicals of Echinacea purpurea (Echinacea purpurea nanoparticles or EPNs)⁽⁵⁾. The physiochemical characteristics of these nanoparticles, enhanced surface area, and increased reactivity provide a means of marking an enhanced biological effect. Reports in recent studies suggest that EPNs can easily cross barrier cells for the active release of constituents in the presence of ROS, allowing for boosting antioxidant activity while reducing potential systemic side effects due to non-target effects⁽⁶⁾.

EPNs have demonstrated a promise in neutralising free radicals and modulating inflammatory pathways by interfering with cellular signalling mechanisms concerned with oxidative stress⁽⁷⁾. These nanoparticles could provide novel therapeutic options for the prevention and management of diseases linked to oxidative harm. Also, their improved bioavailability makes them good candidates for oral medicinal applications, thereby overcoming some of the limitations associated with traditional formulations of Echinacea^(8,9).

The specific objectives of this study bring forth the antioxidant effects of Echinacea purpurea using in vitro and in vivo experimental approaches. It focuses on the mechanisms of action at the cellular level on the effects of ROS neutralisation and inflammatory pathways to provide evidence for EPNs as a natural antioxidant for oral medicine use. Traditional herbal practices and modern nanotechnology are combined to draw attention to the pharmacological uses of EPNs and initiate their clinical applicability against oxidative stress conditions.

Materials & Methods

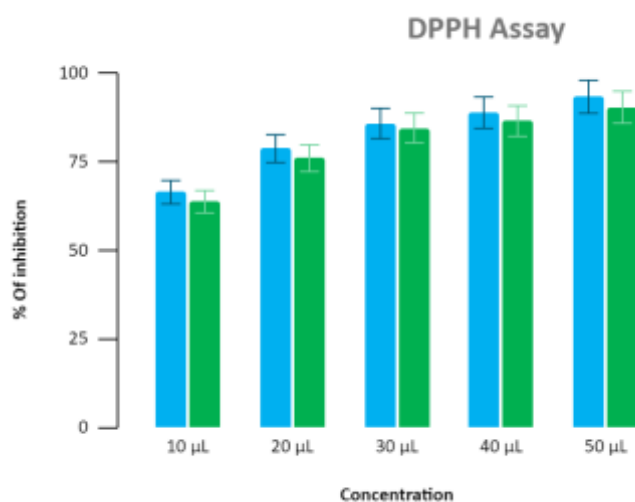
The plant material for the study consisted of dried leaves and/or roots of Echinacea purpurea, either obtained from certified suppliers or grown in a controlled environment to maintain consistency and quality. The antioxidant assay began with chemical materials such as methanol or ethanol (analytical grade) for extraction, DPPH (2,2-diphenyl-1-picrylhydrazyl) was the radical source, and ascorbic acid was the standard antioxidant. Other reagents used to prepare solutions included sodium hydroxide (NaOH), hydrochloric acid (HCl), and distilled water.

DPPH assay

The antioxidant activity of Echinacea purpurea nanoparticles was evaluated via the DPPH radical scavenging assay. For the preparation of the DPPH solution, a 0.1 mm solution was prepared in methanol. Scavenging efficiency was determined by preparing solutions of different concentrations of nanoparticles (10, 25, 50, 75, and 100 µg/ml).

In the experiment, 2 ml of DPPH solution was mixed with different concentrations of the nanoparticle solutions in the test tubes. The reaction mixtures were then incubated in the dark at room temperature for 30 minutes to allow interactions between the DPPH radicals and the nanoparticles. After the incubation, the absorbance of each of the mixtures was read at 517 nm using a UV-Vis spectrophotometer to quantify their radical scavenging activity. Antioxidant activity was calculated as follows: Scavenging activity (%) = {(absorbance of the control (DPPH solution without nanoparticles) - absorbance of the sample (DPPH solution with nanoparticles)) / absorbance of the sample (DPPH solution with nanoparticles)} * 100. The absorbance of the sample (DPPH solution with nanoparticles) indicates how much scavenging activity has occurred. The percentage of scavenging activity represents the extent to which Echinacea purpurea nanoparticles would be effective in neutralizing free radicals. This method serves as a reliable procedure to test the antioxidant efficacy of nanoparticles derived from Echinacea purpurea for their potential applications in oral medicine.

RESULTS



The DPPH assay graph represents a method to assess the antioxidant activity of various samples based on their ability to neutralize free radicals. It compares percentage inhibition at different concentrations (from 10 to 50 µL) for two groups: the standard antioxidant (blue bars) and Echinacea Purpurea Nanoparticles (green bars). Percentage inhibition is a measure of how much a sample may be able to scavenge free radicals, thus giving an idea about the antioxidant potential. A 10-50 µL concentration increment led to an increase in the % inhibition of Echinacea Purpurea Nanoparticles as well as that of the standard. This indicated that with higher concentration nanoparticles, free radicals are being scavenged more efficiently. Contrarily, at lower concentrations (just around 10-20 µL); the standard has shown little more antioxidant activity than the nanoparticles; however, at higher concentrations (30-50 µL), the gap between the two is slowly closing, indicating that Echinacea Purpurea Nanoparticles can achieve similar antioxidant efficacy as the standard.

Data variability is displayed in the error bars in the figure, representing both groups' minor discrepancies, implying fantastic reliability and reproducibility of the results. The results show that Echinacea Purpurea Nanoparticles have good antioxidant activity, which increases with concentration to comparable levels with a standard well-known antioxidant.

This also indicates their viability in possible oral medicinal applications, especially concerning diseases that may bestow oxidative stress.

DISCUSSION

The antioxidantability of nanoparticles of *Echinacea purpurea* has attracted much attention for their better bioactivity and stability over their conventional extracts. Scavenging free radicals, chelating metal ions, up-regulating endogenous antioxidant enzymes, and inhibiting lipid peroxidation further make these nanoparticles promising candidates for various biomedical and pharmaceutical applications.

Many studies indicate that free radical scavenging was significantly aided by the phenolic compounds, flavonoids, and alkaloids of *Echinacea purpurea* (10). The activity was extensively evaluated by the DPPH radical scavenging assay, with nanoformulated *Echinacea purpurea* being found as more superior than its crude extracts (11). Such improvement was credited to the increased surface area and bioavailability of nanoparticles that are more potent in neutralizing ROS (12,13).

Nanoparticles of *Echinacea purpurea* contain polyphenolic compounds that will exhibit metal ion chelation properties, thus reducing catalytic activity of metal ions like Fe^{2+} Cu^{2+} in contributing to oxidative stress via Fenton reaction (14). This feature gains importance in neurodegenerative diseases, as metal-induced oxidative damage becomes a potent pathogenic factor (15). Research shows that *Echinacea*-silver nanoparticles (AgNPs) are potent in metal chelation that reduces neurotoxicity and protects neuronal cells from oxidative damage (16).

According to some studies, nanoformulations of *Echinacea purpurea* can stimulate the antioxidant defense system of the body by upregulating several key antioxidant defense enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (17). Such enzymes serve an important role in detoxifying reactive oxygen species (ROS) and reducing oxidative stress-related damage at the cellular level (18). The upregulation of SOD and GPx expression in neuronal model conditions indicates that *Echinacea* nanoparticles have neuroprotective potential, especially in the context of diseases such as Alzheimer's and Parkinson's disease (19).

Lipid peroxidation plays a lead role in cellular damage via oxidative stress and is said to compromise integrity of membranes and mitochondrial functionality. Nanoparticles of *Echinacea purpurea* have been discovered to inhibit lipid peroxidation, thus maintaining membrane integrity and preventing apoptotic cell death (20). Investigations into dermatological applications show that those nanoparticles, indeed, afford UV protection and anti-aging benefits, supporting their use in skin care formulations (21,22,23).

Nanoparticle formulation extracts of *Echinacea purpurea* are directly compared with extracts as such and have been found to provide better stability and increase ROS neutralization with greater protection offered at cellular level. This whole bioavailability and therapeutic efficacious optimization also suggests that nanoparticle-based formulations could be more clinically effective than ordinary extracts (24).

This vast-antioxidant potentiality of nanoparticles extracted from *Echinacea purpurea* can be extended towards medical, pharmacological, dermatological, nutraceutical, and environmental spheres. Because of their neuroprotective, anti-inflammatory, and immune-modulating properties, they provide a fertile ground for the development of new therapeutic agents for oxidative-stress-related disorders, stimulating skin conditions, and functional food formulations (25). Their potential inclusion in eco-friendly formulations for pollution control and water purification makes them all the more relevant in industrial applications (26).

CONCLUSION

Enhanced antioxidant properties of nanoparticles of *Echinacea purpurea* enable them to be considered to be a promising natural therapeutic agent. These nanoparticles exhibit free radical scavenging activity, heavy metal ion chelation, upregulation of endogenous antioxidant enzymes, and inhibition of lipid peroxidation, thereby leading to enhanced bioactivity. Further studies in the form of clinical trials and mechanistic studies should be conducted to establish their efficacy in disease prevention and treatment.

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