

Echinacea purpurea L. (Moench) Hemagglutinin

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Contributor: Gabriele Balciunaite-Murziene

Echinacea purpurea L. (Moench) is used in traditional and conventional medicine. However, there is lack of data on the biological activities of primary plant metabolite lectins. The aim of our experiment was to find out how lectin LysM (lysine motif), which was previously purified, affects the immune response in vivo. Eight-week-old BALB/c male mice (n = 15) received four weekly 250 µg/kg peritoneal injections of purified *Echinacea purpurea* L. (Moench) roots' LysM lectin. The control animal group (n = 15) received 50 µL peritoneal injections of fresh *Echinacea purpurea* L. (Moench) root tincture, and the negative control animal group (n = 15) received 50 µL peritoneal injections of physiological solution. At the fifth experimental week, the animals were sedated with carbon dioxide, and later euthanized by cervical dislocation, and then their blood and spleen samples were collected. The leukocytes' formula and lymphocytes' count was estimated in blood samples, the T lymphocytes' density was evaluated in spleen zones. A statistically significant ($p < 0.05$) difference between each group was observed in the leukocytes' formula (monocytes' percentage, also little, medium and giant size lymphocytes). The purple coneflower fresh roots' tincture significantly decreased ($p < 0.05$) the T lymphocytes' quantity in peritoneal lymphoid sheaths (PALS) compared with the physiological solution injection's group ($p < 0.05$) and the lectin injection's group ($p < 0.001$). Meanwhile, lectin injections caused a significant ($p < 0.01$) increase in the T lymphocytes in a spleen PALS zone, compared with the physiological solution and tincture injection's group. Our data suggests that LysM lectin acts as an immunostimulant, while fresh purple coneflower tincture causes immunosuppression.

Keywords: *Echinacea purpurea* L. (Moench) ; lectin ; hemagglutinin ; LysM ; immune response ; T lymphocytes ; spleen ; blood cells

1. *Echinacea purpurea* (L.) Moench

Echinacea purpurea (L.) Moench is an endemic plant in the U.S. Great Plains and the Canadian prairies of North America. The indigenous traditional healers from North American tribes of Cheyenne, Choctaw, Dakota, Delaware, Fox Kiowa, Montana, Omaha Pawnee, Ponca, Sioux, and Winnebago indicated *Echinacea* preparations for the healing of wounds, skin inflammatory conditions, infectious diseases, coughs and chest conditions, sore throat and thrush, and also as an antidote against snakebites and poisons, and as a pain reliever ^{[1][2]}. Even though *Echinacea* originates in North America, the traditional knowledge of this plant's beneficial effects was introduced to European settlers and it has spread beyond the Atlantic Ocean. In modern times, the plant's traditional description was changed from "anti-infective" to immunomodulatory active ^{[3][4]}. It is worth to mention that the immunomodulatory activity might be related to antimicrobial and antiviral activities due to the synergistic effect on the host immune system ^{[3][5]}. The immune-enhancing effects of *Echinacea purpurea* (L.) Moench preparations are well documented and the underlying mechanisms have been widely investigated ^{[6][7][8][9]}. The plant's important components are caffeic acid derivatives, alkylamides, flavonoids, polysaccharides, lipoproteins and polyacetylenes. Among them, the caffeic acid derivatives and alkylamides were proved to have effects of immunoregulation ^[10]. At their highest concentration, polysaccharides are typically present in aqueous or fresh pressed juice extracts, while alkylamides (as major constituents) are more likely to be found in ethanolic extracts ^[10].

2. Hemagglutinins (lectins)

Hemagglutinins (lectins) are natural bioactive proteins and glycoproteins that have the capability to specifically bind sugars ^[11]. These sugar-binding proteins are of non-immune origin, and can agglutinate cells or precipitate glycoconjugates ^[12]. Previously, numerous lectins have been isolated from various sources such as plants, algae, and fungi. Plants' lectins are known to have immunomodulatory activity. They are capable of modulating the secretion of cytokines and the production of other immune mediators, such as reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI), in order to improve the host's resistance to microbial infections ^[13]. Plants' lectins have the ability to bind to specific carbohydrate residues, which are present in the membrane of both the bacterial and immune cells of the host. Plants' lectins are mitogens that have the ability to stimulate lymphocytes to undergo mitosis in a calcium-dependent

manner ^[11]. This stimulation is a result of the interaction between the plants' lectins and the surface's sugar moieties present on the surface of lymphocytes. T lymphocytes' cell proliferation is an essential factor to monitor the different adaptive immune reactions. In a variety of studies, authors used spleen, mainly of mice, to investigate the mitogenic power of lectins. Splenocytes encompass macrophages, dendritic cells, and B and T cells; these splenic populations are capable of generating innate and adaptive immune responses ^[10].

Lactose-, D-mannose-, and D-galactose-specific glycoproteins (~40 kDa) were extracted and purified from fresh *Echinacea purpurea* (L.) Moench root. The purified glycoproteins were identified by LS-MS/MS as a two LysM (lysine motif) domain that contains lectins. The LysM domain, which contained lectins, demonstrated hemagglutinating activity ^[14]. There are no data on *Echinacea purpurea* (L.) Moench's lectin effect on the immune response in vivo. Therefore, in the present study we investigated the effects of *Echinacea purpurea* (L.) Moench (EP) fresh roots' tincture, and purified (from *Echinacea purpurea* (L.) Moench fresh root) glycoproteins with the LysM domain in a mouse model by analyzing samples of blood and spleen.

3. Conclusions

To sum up, lectins from purple coneflower fresh roots induced the secondary immune response in the BALB/c mice experimental model in vivo. These results can be supported by a statistically significant neutrophil and monocyte quantity decrease and a statistically significant lymphocyte quantity increase in the blood samples of mice. The vacuolated neutrophils' quantity could have also been increased because of the lectins' interaction with the plasmic membranes of the mentioned cells. The statistically significantly higher medium-sized lymphocyte quantity in the blood samples, and the T lymphocyte count in the mice spleens' PALS zones indicates augmentation of the specific immune response.

Purple coneflower fresh roots' ethanolic tincture (1:5) causes immune suppression of the specific immune response but has a tendency to induce a primary immune response.

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