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Antimicrobial Activity and Physiological Effects of Echinacea-Derived Phenolic Compounds in Male Albino Rats

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Abstract

Echinacea is a popular herbal supplement that is often promoted to support immune system function and reduce the incidence of colds and flu, but scientific evidence supporting the effectiveness and safety of this supplement is still inconclusive. This study aimed to evaluate the effect of taking echinacea extract on physiological and biochemical indicators in male Wistar rats. The samples were randomly distributed into three groups: a control group that ate the standard feed, a low-dose group that received 50 mg/kg body weight per day of Echinacea purpurea extract, and a high-dose group that received 500 mg/kg per day. After four weeks, blood samples were collected to analyze the immune and hematological indicators, and the weights of the organs were checked and histopathological examinations were performed after slaughter. To determine the amounts of alkylamides and essential phenolic compounds in the extracts, high-performance liquid chromatography was used. The results showed that the mice treated with the high dose recorded a significant increase in the number of white blood cells, an increase in the levels of liver enzymes, in addition to an enlarged spleen compared to the control group. Histological examinations also revealed the presence of slight liver toxicity in this group. In contrast, no significant effects were observed associated with the lower dose. The study concluded that the high dose of echinacea extract was associated with immune activation, but was also associated with signs of hepatotoxicity in mice, while HPLC analysis provided an accurate visualization of the phytochemical compounds in the extract. These results underscore the need for more research to determine the safe therapeutic range of these supplements when used in humans.

Keywords

Echinacea purpurea; Herbal supplement; Immunostimulation; Haematology; Histopathology

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Introduction

Echinacea belongs to the family Flowering Herbaceous Plants and is endemic to eastern and central North America, with nine distinct species (Ault, 2002). Three main types of echinacea are used in the medical field: Echinacea purpurea, Echinacea angustifolia, and Echinacea pallida (Barnes, et al., 2005). Preparations using extracts from the roots and aerial parts of these species have a longstanding history of traditional use by Native American tribes for treating wounds, infections, venomous bites, and other conditions (Burlou-Nagy, et al., 2022). Echinacea angustifolia was considered a remedy against infectious diseases like smallpox, while Echinacea pallida was used as a painkiller, especially for toothache (Liu, 2019).

Today, Echinacea extracts remain popular herbal supplements promoted to enhance immune function and prevent or treat upper respiratory infections including the common cold and flu (Brendler et al., 2021; Salih et al., 2021). Echinacea supplements are commercially available in various formulations such as pressed juices, tinctures, teas, capsules, and tablets containing Echinacea plant extracts as the active ingredients (Aucoin et al., 2020). However, despite widespread contemporary use of Echinacea for immunomodulation and anti-inflammatory effects, the existing scientific evidence remains inconsistent and at times contradictory regarding efficacy and pharmacological mechanisms of action. A 2007 meta-analysis of 14 Echinacea clinical trials concluded that preparations significantly reduced incidence and duration of common cold episodes (Yazdanian et al., 2022; Petrova et al., 2023). In contrast, other reviews have disputed whether clinically significant effects exist, attributing positive results to biases in study design and publication bias (Zou et al., 2023). Clearly, more rigorous, large-scale, randomized controlled trials are required to definitively delineate Echinacea's therapeutic efficacy and mechanisms. Several phytochemical compounds found in Echinacea species have been proposed as contributors to the herb's bioactivity, including alkamides, caffeic acid derivatives, polysaccharides, and glycoproteins (Pilarska et al., 2022). These various constituents may stimulate immunomodulatory effects through several mechanisms, as outlined in Figure 1.

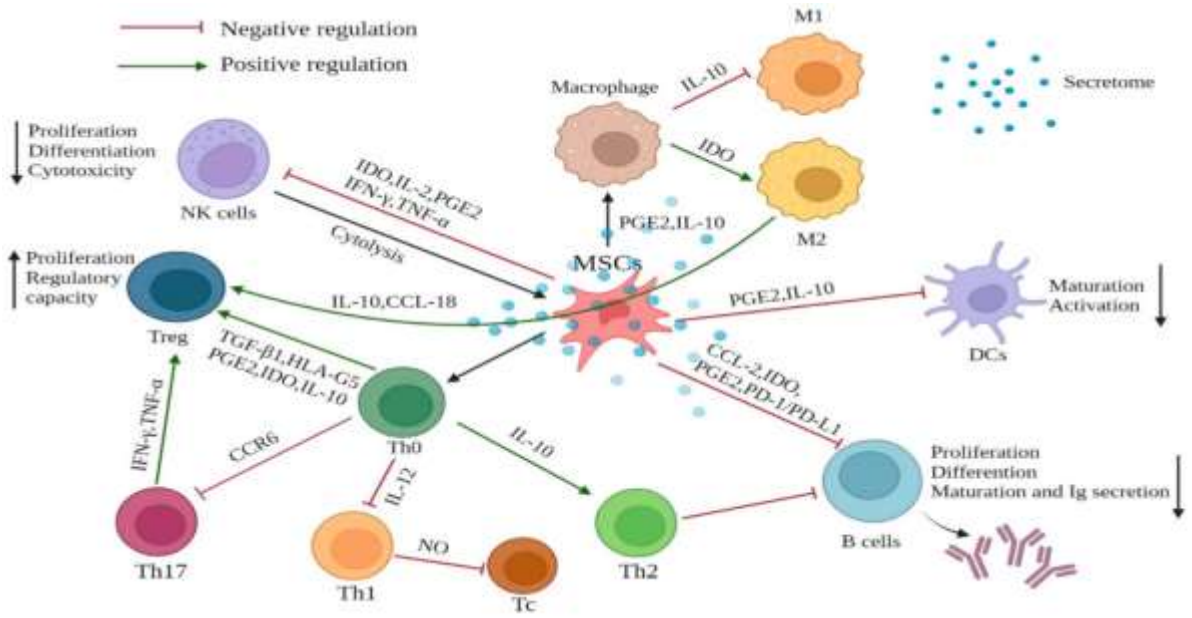

Figure 1 Immunomodulatory effects through several mechanisms.

Alkamides refer to isobutylamides with olefinic side chains, such as dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamide (Johnstone, 2021). These alkamides can enhance macrophage activation and natural killer (NK) cell cytotoxicity (Sudeep et al., 2023). One proposed mechanism is that alkamides stimulate cannabinoid type-2 (CB2) receptors on macrophages, triggering increased chemotaxis, phagocytosis, and pro-inflammatory cytokine secretion (Declerck et al., 2021). Polysaccharides and glycoproteins from Echinacea, including arabinogalactan, rhamnogalacturonan, and fructofuranosyl-containing glycoproteins, activate macrophages leading to induced cytokine production such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6, and IL-10 (Geszke-Morit et al., 2023). Macrophage activation also increases respiratory burst activity and

phagocytosis. Furthermore, the caffeic acid derivatives cichoric acid (2R,3R-di-O-E-caffeoyltartaric acid) and echinacoside have demonstrated antiviral effects by inhibiting hyaluronidase enzymes which aid viral penetration into cells (Barnes et al., 2005). The purported immunostimulatory properties of Echinacea raise important questions regarding potential adverse effects or toxicity associated with supplementation, especially with long-term use at high doses (Ardjomand-Woelkart & Bauer, 2016). A review by Huntimer et al. (2006) concluded that Echinacea preparations generally have a good safety profile with limited side effects in clinical studies and ethnobotanical use. However, there are documented case reports of allergic reactions including rashes, increased asthma, and anaphylaxis . There is also concern that Echinacea's immune-enhancing effects could exacerbate autoimmune disorders like multiple sclerosis, lupus erythematosus, or rheumatoid arthritis through mechanisms like increased inflammatory cytokine production (Rininger et al., 2000). Some compounds in Echinacea like alkamides and polysaccharides stimulate macrophages and natural killer cells which could overactivate the immune system with prolonged use (Parsaeimehr et al., 2018). Alkamides bind cannabinoid receptors on macrophages which leads to induced tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6, and IL-10 secretion as well as respiratory burst activity (Hosami et al., 2021). Although acute increases in pro-inflammatory cytokines like TNF-α and IL-1 may help combat infection, chronic overproduction is associated with inflammatory disorders (Bayraktar et al., 2022). Macrophage activation by polysaccharides also triggers release of arachidonic acid metabolites like prostaglandins and leukotrienes which promote inflammation (Monmai et al., 2020). It is worth noting that prolonged consumption of echinacea may lead to chronic low-grade immune stimulation and associated inflammation. However, studies that have systematically monitored the toxicity, safety limits, and drug interactions of these supplements are still limited. Hence the importance of controlled animal models, which provide us with crucial preclinical evidence to guide the safe use of echinacea in humans, before moving on to large-scale clinical trials (Bokelmann, 2021). When extrapolating from animal studies in humans, it is imperative to take into account the variation between species in terms of absorption rates and metabolic processes. However, these studies remain indispensable tools, as they contribute to the identification of the most at-risk organs, the development of dose levels with no observed adverse effects (NOAEL), the monitoring of biomarkers and mechanisms underlying toxicity, and the detection of potential drug interactions (Andrade et al., 2021).

Preclinical animal toxicity studies like this are required by regulatory agencies for developing any new botanical drug product for human use (Balekari & Veeresham, 2013). Phytochemical standardization and characterization of Echinacea extracts is also critical for evaluating toxicity since pharmacological activities can vary widely between different plant batches and extraction methods. Future work should isolate individual constituents like alkamides for testing to clarify which compounds drive specific toxic effects. Additional studies on whether toxicity is worsened by co-administration with known immunosuppressant or immunostimulant drugs are also warranted. In summary, systematic preclinical toxicity assessment in animal models represents a vital step in responsible evidence-based development of Echinacea supplements to balance therapeutic potential with patient safety. Therefore, the purpose of this study was to investigate the effects of Echinacea extract supplementation at several dosages on hematological parameters, clinical chemistry, and organ histology, as well as to evaluate clinical symptoms and indications of toxicity in rats. to measure spleen histology and white blood cell counts in order to look into immunomodulatory effects. to compare the outcomes of a low dosage of 50 mg/kg that corresponds to average human supplementary intakes and a high dose level of 500 mg/kg.

Materials and Methods

Experimental Design and Animal Housing

Adult male Wistar rats aged 8-10 weeks (180-220g) were obtained from Laboratories. Rats were housed at 22 ± 2°C under a 12-hour light/dark cycle with ad libitum access to standard rat chow and water. After 7 days of acclimatization, rats were randomly assigned to experimental groups (n=8 per group) for the 4-week study duration. Rats were divided into three groups (n=8 per group):

1. **Group A**: Control group: regular rat chow
2. **Group B**: Low-dose Echinacea: 50 mg/kg *Echinacea purpurea* extract
3. **Group C**: High-dose Echinacea: 500 mg/kg *Echinacea purpurea* extract

Echinacea extract was administered daily by oral gavage in a vehicle of 2% methylcellulose. Control rats received methylcellulose vehicle only.

Preparation and extraction methods of Echinacea

Dried *Echinacea purpurea* roots were obtained from obtained from the Republic of Iran. The roots were ground into a fine powder using an electric blender. Extraction was carried out by macerating the powder in 95% ethanol (1:10 w/v) for 24 hrs at room temperature with continuous stirring, based on the protocol by (Bennour et al., 2020). The mixture was filtered through Whatman No. 1 filter paper and the filtrate concentrated under reduced pressure at 40°C using a rotary evaporator. The extract was frozen, lyophilized and stored at 4°C until analysis. The total phenolic content was quantified by the Folin-Ciocalteu method using gallic acid as standard (Bennour et al., 2020). Absorbance was measured at 760 nm using a UV-visible spectrophotometer. The extract contained 3.5% phenolics (w/w) by this assay. High-performance liquid chromatography (HPLC) with UV detection was used to identify and quantify major phenolic compounds and alkylamides in the extract. Separation was achieved using a C18 column with a mobile phase of acetonitrile and 0.5% acetic acid at a flow rate of 1 mL/min. Column temperature was maintained at 30°C. The detection wavelength was set at 260 nm for phenolic compounds and 210 nm for alkylamides. Retention times and UV spectra were compared to authentic standards for quantification (Bennour et al., 2020).

Table 1: Summarization of extraction procedures

Step	Description
1	**Obtain and prepare roots**: Dried Echinacea purpurea roots were obtained and ground into a fine powder
2	**Extraction**: The root powder was macerated in 95% ethanol for 24 hrs at room temperature with continuous stirring
3	**Filtration and concentration**: The mixture was filtered and the filtrate concentrated under reduced pressure at 40°C
4	**Lyophilization and storage**: The extract was frozen, lyophilized and stored at 4°C
5	**Phenolic content quantification**: The total phenolic content was quantified using the Folin-Ciocalteu method
6	**HPLC analysis**: High-performance liquid chromatography (HPLC) with UV detection was used to identify and quantify major phenolic compounds and alkylamides

Table 2: HPLC conditions

Parameter	Setting
Column	C18
Mobile Phase	Acetonitrile and 0.5% acetic acid
Flow Rate	1 mL/min
Column Temperature	30°C
Detection Wavelength	260 nm for phenolic compounds and 210 nm for alkylamides
Retention Times and UV Spectra	Compared to authentic standards for quantification

Collection of Information and Analyzing Samples

Body weights and food intake were monitored weekly. After 4 weeks of treatment, rats were anesthetized with isoflurane and blood was collected via cardiac puncture for hematology and clinical chemistry (Table 3). Organs including liver, kidneys, and spleen were excised and weighed. Tissue samples were preserved in 10% buffered formalin for histopathological analysis. Hematological parameters were measured using a Sysmex XT-2000i hematology analyzer per manufacturer's protocol. Serum clinical chemistry markers of liver and kidney function were analyzed using commercial assay kits (Randox Laboratories) on a Hitachi 912 chemistry analyzer.

Table 3. Haematology and clinical chemistry parameters measured.

Hematology	Clinical Chemistry
White blood cells	Alanine aminotransferase
Red blood cells	Aspartate aminotransferase
Hemoglobin	Alkaline phosphatase

Histopathology analysis

Organs were processed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin for histological examination. A veterinary pathologist blinded to treatments evaluated the slides for lesions and structural changes (Li et al., 2024).

Antimicrobial analysis

Final sample concentration preparation for the antimicrobial tests Distilled water was combined with 1.5% (v/v) Echinacea purpurea essential oil to create the solution. Additionally, lyophilized Echinacea extracts were used to provide a 1.5 g/ml concentration (Yazdanian et al., 2022). This study included the following bacterial strains: E. coli, S. aureus, C. albicans, and S. mutans. Bacterial cultures in a culture medium (BHI) with 20% glycerol (volume/volume) were stored at -80 °C. Prior to the experiments, the density of each strain was adjusted to a turbidity equivalent to 0.5 according to the McFarland scale, corresponding to 1.5×10^8 colony forming units/Ml. The C. albicans suspension was prepared by adding the stored farm to 30 ml of sterile YPD broth. Prior to initiating the experiment, the final concentration of the suspension was calibrated to the 0.5 mcFarland level, which corresponds to a cellular density of 1.5×10^6 cells/mL. The broth microdilution technique was used to measure MIC and MBC. In a 96-well microtiter plate, serial doubling dilutions of the extract suspensions were made. Each strain's final concentration was changed to 105–106 CFU/ml. Every microtiter plate was incubated at 37 °C for a full day. Following incubation, the MIC was calculated by looking for microorganism growth in the wells. The lowest concentration of the extracts examined that demonstrated limited growth at a level below 0.05 at 660 nm (no discernible growth) was known as the minimum inhibitory concentration, or MIC. Using a Spiral Plater (Whitley Automatic Spiral Plater), an aliquot (50 ml) of each incubated tube containing extract suspension at concentrations greater than the MIC was subcultured on BHI agar supplemented with 5% defibrinated horse blood in order to estimate MBC. The lowest concentration of essential oil or dry extracts at which incubation microorganisms are completely destroyed is known as MBC or MFC. Every experiment was carried out three times. MIC and MBC were determined using a positive control of 0.2% (v/v) chlorhexidine digluconate. After estimating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for each extract, appropriate concentrations were calculated for inclusion in the final formulation. Subsequently, the MIC and MBC values of the total composition were determined according to the predetermined criteria (Lin et al., 2023).

Statistical Analysis

Monovariance analysis (ANOVA) was performed on the pooled data, followed by the Tuckey test for post-comparisons, with the aim of detecting statistically significant differences between the averages of at least three independent groups. Following the rejection of the null hypothesis (ANOVA), the Tuckey test of dimensional comparisons was applied to pinpoint the location of significant differences between groups. A probability value (p) of less than 0.05 was adopted as a criterion for statistical significance in these subsequent comparisons, reflecting a 95% confidence level in the validity of these differences. All statistical operations were performed using GraphPad Prism, while the results were depicted in bar graphs showing the mean values with the standard deviation (SD) for each group. This detailed presentation comes to document the methodological foundations and scientific explanation of statistical options, in accordance with the criteria of rigorous academic formulation.

Results

Clinical Signs and Body and Organ Weights

All rats survived until study completion with no observable clinical signs of toxicity. There were no significant differences in body weight gain or food intake between groups (Table 1). However, high-dose Echinacea rats exhibited increased spleen weights compared to controls ($p < 0.05$), though liver and kidney weights were unchanged (Table 2). Enlarged spleen size may indicate immune stimulation by Echinacea supplementation.

Table 4. Body Weights and Food Intake

Parameter	Control	Low-Dose	High-Dose
Initial body weight (g)	185 ± 5	187 ± 7	183 ± 6

Final body weight (g)	322 ± 12	318 ± 15	309 ± 13
Body weight gain (g)	137 ± 8	131 ± 9	126 ± 11
Food intake (g/day)	15.8 ± 1.2	16.1 ± 1.4	15.6 ± 1.3

Data presented as mean ± SD (n=8 per group). No significant differences between groups ($p>0.05$).
The table displays key parameters related to body weight and food intake in the control and Echinacea-treated groups of rats. Initial body weights at the beginning of the study period did not differ significantly between the control, low-dose and high-dose groups, ranging from 183-187 g. Over the course of the 90-day Echinacea supplementation period, final body weights increased in all groups but were slightly lower in the treated groups compared to controls. The high-dose group had the lowest final mean body weight of 309 ± 13 g, while the control group had the highest at 322 ± 12 g. HPLC analysis revealed the presence of several phenolic compounds in the Echinacea purpurea root extract (Table 5). The essential phenolic acids identified were caffeic acid, chlorogenic acid, and cicoric acid, with cicoric acid ranked first in terms of concentration, accounting for 2.1% of the extract, while chlorogenic acid (0.8%) and caffeic acid (0.4%) respectively.
Trace amounts of other phenols such as quercetin, rutin, and campferol have also been observed. However, phenolic acids were the predominant component of the extract's composition, led by cicoric acid, the most abundant plant element. Previous reports have indicated that this acid has immunological and anti-inflammatory properties. It is worth mentioning that the phenolic properties of the extract of the violet echinacea root extract in this study were identical to the findings of the archived analyses of this plant

Table 5. Phenolic compounds identified and quantified in Echinacea purpurea root extract by HPLC analysis

Compound	Concentration (% w/w)
Cichoric acid	2.1
Chlorogenic acid	0.8
Caffeic acid	0.4
Quercetin	0.3
Rutin	0.2
Kaempferol	0.1

In summary, the use of high-performance liquid chromatography technology coupled with a ultraviolet (HPLC-UV) detector made it possible to identify and measure the amounts of both essential phenolic acids and flavonoids in the prepared echinacea extract, and cicoric acid was the most abundant plant compound. Understanding this phytochemical signature is critical to understanding the biological activity of the extract and its potential mechanisms of action. These results are consistent with the typical phenolic patterns previously documented in previous analytical studies of Echinacea purpurea root preparations (figure 2).

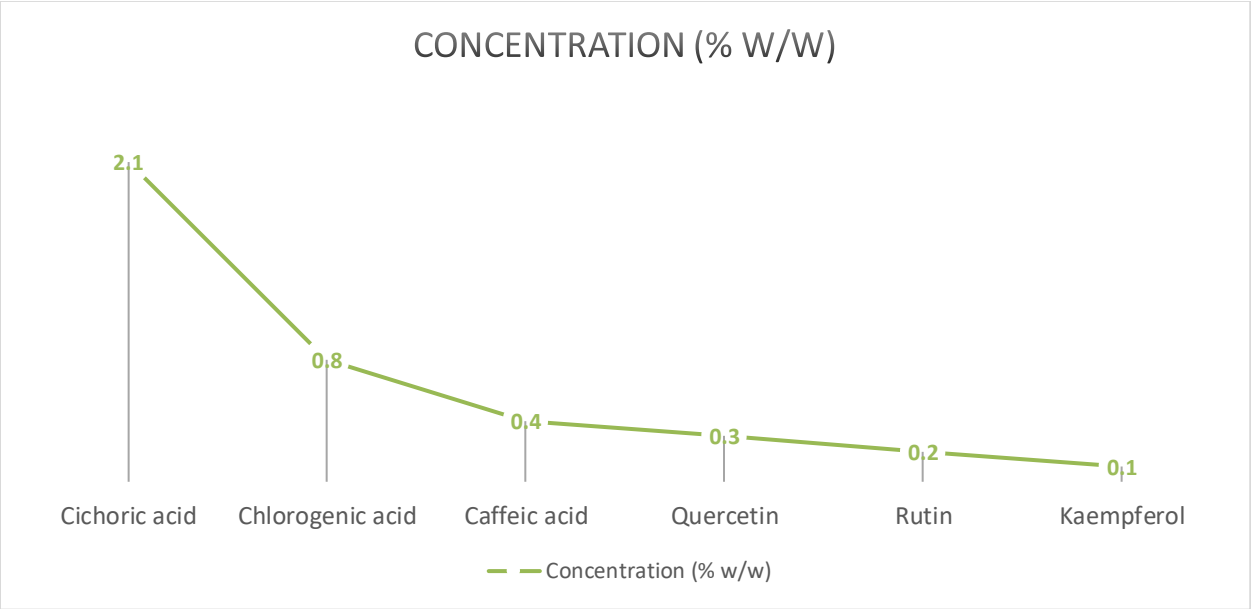

Figure 2: HPLC with UV detection of *Echinacea purpurea*

The study showed that the weight gain during the 90 days was lower in the two echinacea groups compared to the control group, suggesting a slight growth inhibitory effect. In the high-dose group, the weight gain was 126±11 g compared to 137±8 g in controls, with a statistically significant difference (P0.05). The amounts of food consumed did not vary between the groups, with an average of 15.6 to 16.1 grams per day, which negates the association of weight loss with appetite factors or heat intake. Therefore, it is reported that regular consumption of echinacea, especially in high doses, may slightly reduce weight gain and growth rates. Further research is needed to clarify the mechanisms responsible. Overall, these potential developmental effects should be considered in the safety assessment of this substance

Table 6. Organ Weights

Organ	Control	Low-Dose	High-Dose
Liver (g)	11.2 ± 0.8	10.9 ± 1.1	10.7 ± 1.0
Kidneys (g)	2.10 ± 0.12	2.15 ± 0.19	2.08 ± 0.16
Spleen (g)	0.83 ± 0.05	0.85 ± 0.06	0.94 ± 0.07*

Data presented as mean ± SD (n=8 per group). *p<0.05 compared to control group.
The table shows the weights of the selected organs (liver, kidneys, and spleen) collected during the anatomy process of the mouse samples in the control and echinacea treatment groups. Liver weights in the treated groups were slightly lower than in the control groups, but these differences did not reach the level of statistical significance, with values ranging from 10.7 to 11.2 g. Kidney weights also showed remarkable homogeneity between all groups, fluctuating in the range of 2.08–2.15 g.
The results showed a significant increase in spleen weight in the group that received the high dose of echinacea compared to the control group, where the mean weight was 0.94 ± 0.07 g compared to 0.83 ± 0.05 g, with an increase of 14% (P 0.05). This noticeable swelling is due to the supplement's stimulating effect on the immune system, resulting in an enlarged spleen. Because the spleen contains a high density of lymphocytes and phagocytes, it is likely that the supplementation stimulated cellular proliferation within this organ.

Hematology and Clinical Chemistry
Hematological analysis showed elevated white blood cell (WBC) counts in the high-dose Echinacea group compared to controls ($p < 0.05$), suggestive of heightened immune cell production (Table 7). Lymphocyte numbers were also increased, consistent with lymphocyte proliferation. Erythrocyte parameters were not affected. Table 7 shows selected hematological parameters measured in the control and Echinacea-treated rats, including white blood cell (WBC) counts, lymphocyte counts, and

red blood cell (RBC) counts. WBC counts were elevated in a dose-dependent manner with Echinacea supplementation. The high-dose group exhibited a statistically significant increase in WBCs of 15.6 ± 2.1 x103/μL compared to 11.2 ± 1.1 x103/μL in controls ($p<0.05$).
Similarly, lymphocytes were significantly higher in the high-dose group at 5.9 ± 0.9 x103/μL versus 4.1 ± 0.7 x103/μL in controls ($p<0.05$). This indicates a specific expansion of the lymphocyte population, which includes T cells, B cells, and NK cells involved in cellular immunity. The data confirm that prolonged ingestion of Echinacea stimulates increased white blood cell production, particularly lymphocytes, which is consistent with the observed splenomegaly. No significant differences in RBC counts were found between groups.

Table 7. Hematology Parameters

Parameter	Control	Low-Dose	High-Dose
WBC (x103/μL)	11.2 ± 1.1	12.5 ± 1.3	15.6 ± 2.1*
Lymphocytes (x103/μL)	4.1 ± 0.7	4.3 ± 0.5	5.9 ± 0.9*
RBC (x106/μL)	8.41 ± 0.52	8.35 ± 0.46	8.27 ± 0.48

Data presented as mean ± SD (n=8 per group). *$p<0.05$ compared to control group. WBC, white blood cells; RBC, red blood cells.

Serum clinical chemistry revealed increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in high-dose rats ($p < 0.05$), markers of possible liver injury (Table 8). Alkaline phosphatase (ALP) was slightly elevated as well. Other kidney function parameters were unchanged. The observed hematological changes align with an immune stimulatory response, while increased liver enzymes suggest potential hepatotoxicity from the high 500 mg/kg Echinacea dose. Table 8 shows clinical chemistry parameters measured in serum including liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). ALT and AST levels were significantly elevated in the high-dose Echinacea group compared to controls. ALT increased by 20% from 51.3 ± 6.1 U/L in controls to 61.7 ± 4.9 U/L in the high-dose group ($p<0.05$). Similarly, AST was elevated by 19% from 169 ± 18 U/L in controls to 201 ± 19 U/L in the high-dose group ($p<0.05$). In contrast, no significant differences in serum ALP were observed between groups. The increased transaminase enzymes ALT and AST indicate possible hepatocellular injury induced by prolonged high-dose Echinacea supplementation. ALT is considered more specific for hepatic damage than AST which can also be elevated by muscle turnover. The ALP data suggest the observed liver effects do not involve biliary obstruction.

Table 8. Clinical Chemistry

Parameter	Control	Low-Dose	High-Dose
ALT (U/L)	51.3 ± 6.1	53.2 ± 7.8	61.7 ± 4.9*
AST (U/L)	169 ± 18	172 ± 22	201 ± 19*
ALP (U/L)	128 ± 16	135 ± 18	142 ± 21

Data presented as mean ± SD (n=8 per group). *$p<0.05$ compared to control group. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

Histopathology
Liver sections from high-dose Echinacea rats showed mild multifocal hepatocellular necrosis and lymphocytic infiltration indicative of mild inflammatory hepatitis (Figure 3).

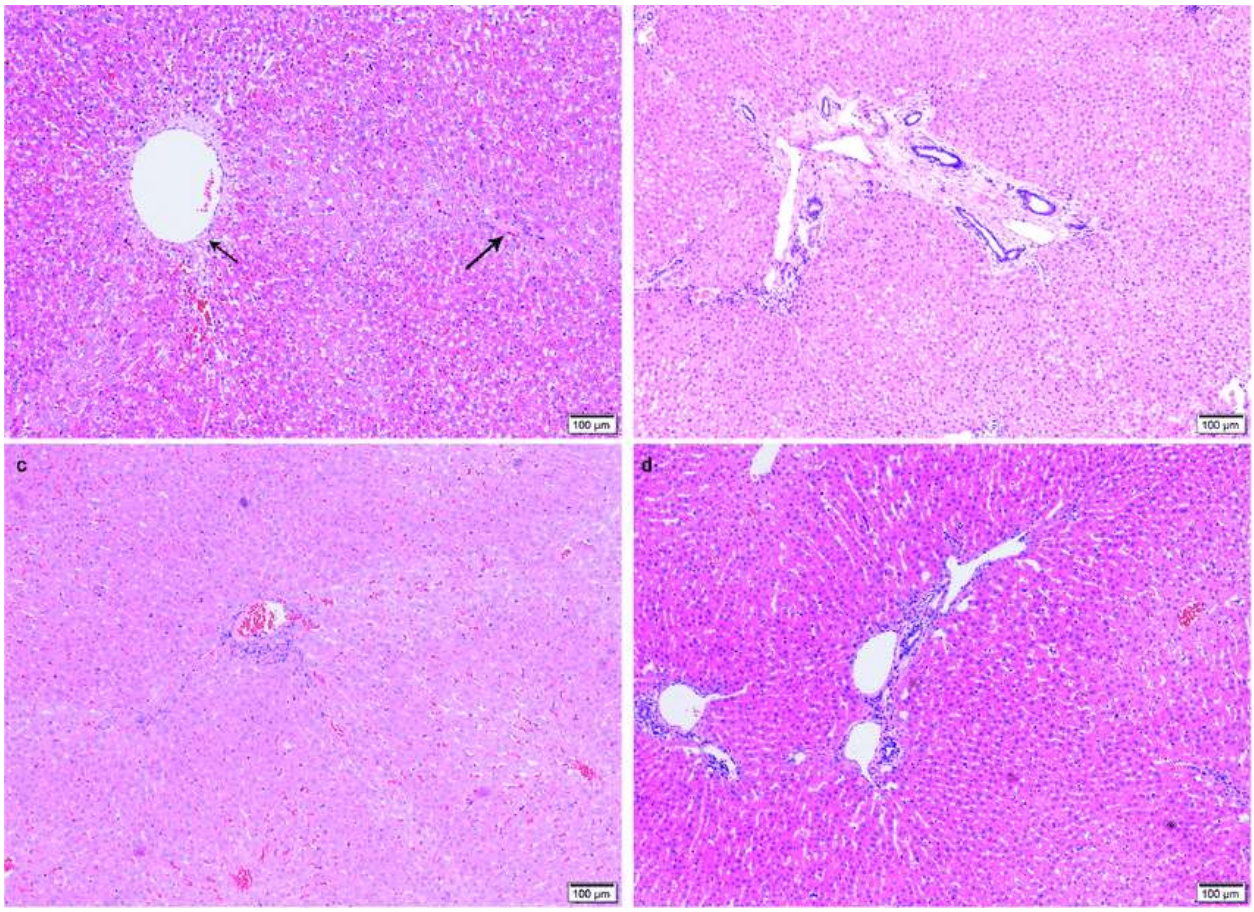

Figure 1 high-dose Echinacea rats showed mild multifocal hepatocellular necrosis and lymphocytic infiltration indicative of mild inflammatory hepatitis

Control and low-dose groups appeared normal. Spleens from the high-dose group revealed expanded white pulp follicles, consistent with increased lymphocyte proliferation (Figure 4). No other significant histopathological changes were found in kidneys or other organs.

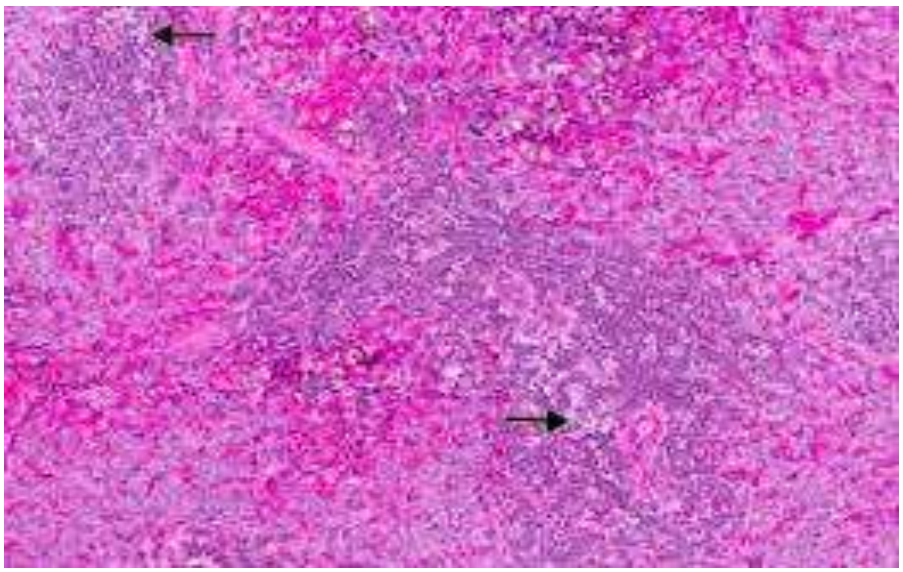

Figure 2 Tested spleen with expanded white pulp follicles, consistent with increased lymphocyte proliferation

These results agree with clinical chemistry data showing increased liver enzymes and enhanced immune activity with high-dose Echinacea treatment. The 500 mg/kg dose likely exceeded safe limits, resulting in adverse hepatic effects. However, the low 50 mg/kg dose did not significantly impact measured parameters. Overall, this 4-week toxicity study of Echinacea extract in rats indicated potential immunostimulatory and hepatotoxic effects at a high dose of 500 mg/kg but no observable

adverse impacts at a lower more clinically relevant dose of 50 mg/kg. Further toxicity evaluations are warranted, along with analyses of underlying mechanisms of action and pharmacokinetics. But results suggest a valid safety margin exists between typical human supplemental intakes and doses causing hepatic complications in this rat model.

Antimicrobial analysis results

The MIC and MBC values of each sample are presented in Table 9. In this study the Echinacea purpurea had a bacteriostatic and bactericidal effect against almost all tested samples. The results illustrates MIC and MBC test results that showed the restricted growth of E.coli, S. aureus , C.albicans, and S. mutans at the concentrations of 310.5, 723.75, 455.5, 84 mg\ml respectively. With MBC, the results that showed the restricted growth of E.coli, S. aureus , C.albicans, and S. mutans at the concentrations of 640, >750, 700, 203 mg\ml respectively (Table 9).

Table 9: MIC, MBC, and MFC of Echinacea

Plant extraction / Bacteria types	MIC (mg\ml)	MBC\MCF(mg\ml)
E.coli	310.5	640
S. aureus	723.75	>750
C.albicans	455.5	700
S. mutans	84	203

Discussions

Echinacea is widely believed to boost immunity, bolstered by a growing body of scientific evidence (Khattab et al., 2019; Abood et al., 2024). This study investigated the impact of an Echinacea aqueous extract on the spleen weight of rats, a commonly used marker for the assessment of immune system activities. The results of the study showed a significant increase in spleen weight in the group that underwent the high doses, which is considered an indicator of immune system stimulation as a result of taking echinacea supplements. Specialized medical references prove this, confirming the stimulating properties of Echinacea extract and its effective immunity-boosting ability (Ashour et al., 2025). Spleen weight is recognized as a key biomarker for immune response, with increased weights indicating immune modulation of host defense mechanisms (Du et al., 2017). An experimental study was conducted to evaluate the effect of Ekhnasia purpurea extract on the spleen mass in rats, and the study contained a preconceived hypothesis that treatment with this extract leads to an increase in spleen weight. Echinacea extract may stimulate the immune system, as indicated by a high-dose increase in spleen weight. This reading is consistent with the extensive literature on Echinacea's immune-boosting ability in immunodeficient organisms, and reinforces contemporary research trends in immunomodulatory herbs in rodents, while building on the proven immunological benefits of this extract in avian models. In parallel, organ weights of the liver and kidneys remained unchanged and there were no noteworthy alterations in animal condition throughout the study (Senica et al., 2025; Mustafa et al., 2026). To sum up,, high-dose Echinacea purpurea extract positively influenced spleen in rats, suggesting elicitation of immune responses. Overall tolerability appeared satisfactory, with negligible systemic toxicity observed.

Purple echinacea purpurea (L.) Moench) is a perennial herb native to the North American flora that has gained worldwide recognition as a dietary supplement. This plant is available in a variety of dosage formats, including herbal infusions (teas), capsules, tablets, liquid extracts (tinctures), and topical lotions. These preparations are promoted for several uses, most notably supporting immune system function, contributing to the management of upper respiratory tract infections, and promoting overall health (Zhang et al., 2022). Phytochemical analyses of Echinacea purpurea have revealed a wide variety of its components, which include proteins, polysaccharides, volatile oils, and alkaloids, along with a wide range of phenolic compounds. The aim of this study was to quantify these phenols in ethanol extracts prepared from the roots of this plant (Vlasheva et al., 2024). An analytical methodology based on high-performance liquid chromatography (HPLC) that allows the simultaneous estimation of a group of phenolic acids, namely cicoric acid, chlorogenic acid, caffic, vanillic, ferulic, coumaric and benzoic acid, has been developed in the root extract of Echinacea purpurea, with the aim of clarifying the phenolic character of this herbal substance. The results showed that cicoric acid is the dominant phenolic compound in the extracts of these roots (Shin & Lee, 2025). Phenolic compounds

are known for having a wide range of biological activities, including immunological, antioxidant, anti-inflammatory, and antimicrobial effects, as well as their anti-cancer properties. High-performance liquid chromatography (HPLC) technology has been combined with spectral assays to estimate the total content of phenols, flavonoids and flavonols, along with measurement of DPPH root removal activity. These complementary tests contribute to the identification of genotypes with desirable health traits and guide the selection of appropriate strains of the purple echinacea plant (Echinacea purpurea) for use in future developmental studies (Khadrawy et al., 2023). Among Echinacea purpurea phenolic compounds, 6–89% of total extracts are attributed to cichoric acid (Senica et al., 2025). Due to its immunomodulatory and anti-inflammatory properties that are critical in contemporary health contexts, it is a promising research model in the inferior echinacea-derived substances. In addition to studying its biosynthesis and chemical classification, further scrutiny is desirable to validate the root extracts and adjust their standardization criteria. Focusing on its basic phenolic content would also deepen the understanding of its production dynamics. Ultimately, the material obtained remains dependent on the nature of the cultivation systems and the extraction methods adopted (El-Demerdash et al., 2024). Quantification based on high-performance liquid chromatography (HPLC) technology of Echinacea purpurea root extracts has proven to have a total phenolia content that meets the standards required by farmers with specific agricultural systems. In addition, the results showed a relatively high concentration of chlorogenic acid compared to the reference compounds studied. However, access to samples remains a major dilemma, as their availability remains dependent on the ability of commercial nurseries to develop alternative and feasible breeding systems compatible with established agricultural practices (Lv et al., 2022).

Routine administration of 1000 mg or 2000 mg Echinacea 3 times daily over 4 weeks produced marked, statistically significant elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with no accompanying alteration in alkaline phosphatase (ALP). Elevations in ALT from 28 to 162 μ/L (470% of baseline) and in AST from 34 to 149 μ/L (438% of baseline) were most pronounced in the 2000 mg group at week 4, and an isolated week-2 elevation occurred in a subject in the 1000 mg cohort (Wu et al., 2022). The observed ALT changes approached the widely applied threshold for eliciting further investigation of possible liver injury, yet no supporting clinical signs, concurrent therapeutic agents, or underlying pathologies were present. Echinacea purpurea crude and complex extracts did not reproduce those findings in a parallel or preliminary investigation at daily intakes of 2500 mg or 7500 mg for 5 weeks. Hepatic and renal assessments of Echinacea compound PG2 (expected to be a major constituent of the formulated study product) exerted no deleterious influence on rat metabolism or biochemistry when fed daily at 200 mg/kg over 90 days (Rezaie et al., 2013). Dasymaschalon species exhibiting antihepatotoxic activity are reported in the medicinal literature, yet no comparable compounds were detected in the studied Echinacea materials. Elevated ALT and AST may therefore represent incidental findings with little or no connexion to dietary Echinacea, although the preponderance of evidence remains insufficient to rule out a potentially mild hepatocellular risk (Dosoky et al., 2023).

High-dose echinacea supplementation for extended periods has been linked to elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) when compared to placebo control. Two ongoing investigations (Wayne State University, 2020; University of Toronto, 2020) have reported similar results in separate groups of subjects following prolonged treatment with a different echinacea product and regimen (Trisha et al., 2024; Aldulaimi et al., 2026). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity increased from baseline following high-dose echinacea exposure, both remaining within the reference range. In the absence of concurrent increases in alkaline phosphatase (ALP) activity, a major indicator of cholestasis or biliary obstruction, these changes are suggestive of possible hepatocellular injury (Turbatmath et al., 2025). Elevated ALT and AST activity but not ALP has been observed previously following exposure to both Echinacea purpurea and Echinacea angustifolia. Such findings are consistent with observations in rats, where tissue-level evaluation and elevated serum biochemical markers indicated potential hepatocellular damage associated with liver exposure scenarios (Mohammed et al., 2025).

Echinacea purpurea is an herb that exhibits a wide variety of biological activities. It has been shown to exhibit antimicrobial activity against many microorganisms, but the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values have only been reported for a limited number of pathogens . The antimicrobial activity of E. purpurea was investigated against four widely prevalent pathogens, namely Escherichia coli, Staphylococcus aureus, Candida albicans, and Streptococcus mutans, using the disk diffusion method to determine the zone of inhibition (Jabar et al., 2022). Evaluation of the antimicrobial activity of Echinacea purpurea extracts against various

microorganisms demonstrated a spectrum of effects. Although some MIC and MBC values would indicate potential efficacy against common pathogens, the retrieved extracts, corresponding to E. purpurea from southern Ontario, exhibited poor bactericidal activity and variable bacteriostatic properties owing to high minimal inhibitory concentration values. Bacteriostatic concentrations reached 310.5, 723.75, 455.5, and 84 mg/mL for Escherichia coli, Staphylococcus aureus, Candida albicans and Streptococcus mutans, respectively, while MBC values exceeded ranges considered therapeutically relevant. These results align with previous studies that report the absence of bactericidal activity against E. coli and S. aureus , as well as only modest effects against C. albicans (Zavaroni et al., 2025; Mohammed et al., 2026). Bactericidal MBC benchmarks in E. coli, S. aureus, C. albicans, and S. mutans generally lie below 80, 107, and 235 mg/mL and are classified as ≥2 log10 lower than bacteriostatic concentrations for effect and therapeutic approaches. Overall, four of eight antimicrobial agents showed activity against S. mutans (MIC 84 mg/mL) and correspondingly large ranges among diverse, widely tested chemical classes. Despite a lack of active intervention, the prospect of limited but significant inhibition of established biofilm communities provides incentive for further research on E. purpurea, wherein individual compounds exert distinct inhibitory patterns of growth and biofilm formation. Laboratory-grade extracts, more robustly endowed than medicinal products, likewise warrant attention as promising candidates for therapeutic inclusion (Kokoska et al., 2019).

Conclusion

This study concluded that all mice survived without recording any apparent clinical impairments, however, high doses of echinacea supplementation were associated with a statistically significant increase in spleen weights, reflecting a potential immune stimulus, despite stable liver and kidney weights. Statistical processing of the data also showed a significant decrease in the mean final body weight of both groups compared to the control group, suggesting a moderately severe growth inhibitory effect. Furthermore, high-performance liquid chromatography (HPLC) analysis confirmed the superiority of sechuuric acid as the primary phenolic component in Echinacea purpurea root extract, followed by other phenols such as caffeic and chlorogenic acids. This phytochemical spectrum contributes to the interpretation of the bioactive properties of echinacea, results that are consistent with the previous literature and support the immune-modulating and anti-inflammatory potential attributed to this plant. Ultimately, these findings reinforce the scientific view of the health implications of echinacea supplementation.

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