



SAFETY EVALUATION OF BURDOCK (*Arctium lappa* L.) ROOT EXTRACT: EFFECTS ON SPLEEN HISTOPATHOLOGY AND PERIPHERAL LYMPHOCYTES IN WISTAR RATS

Imam Agus Faizal^{a*} ; Rananda Afrilla^b ; Meka Faizal Farabi^c ; Lisa Savitri^d

^{a, b, c} Department of Bachelor of Applied Medical Laboratory Technology, Faculty of Pharmacy, Science, and Technology; Al-Irsyad University of Cilacap ; 24 St. Cerme, Cilacap and 53223 ; Indonesia.

^d Department of Medical Laboratory Technology, Faculty of Health Sciences, Universitas Kadiiri; 1 St. Selomangleng, Pojok; Kec. Mojoroto ; Kediri City and 64115 ; Indonesia

Abstract

Arctium lappa L. (burdock) root contains phytochemicals – including flavonoids, saponins, and phenolic compounds – reported to exhibit immunomodulatory activity. This study evaluated the safety of *Arctium lappa* L. root extract through a 28-day post-test experimental design involving male and female Wistar rats. Eight animals were randomized into four groups receiving oral doses of 0, 150, 300, or 450 mg/kg BW/day. Lymphocyte counts were measured using an automated analyzer, and splenic histopathology was assessed on Hematoxylin-Eosin-stained sections using semiquantitative scoring. Lymphocyte values (mean $\pm$ SD) showed no significant differences among groups ($p > 0.05$; small effect size) and remained within physiological limits, with males ranging from 4.14 ± 0.21 to $7.96 \pm 0.34 \times 10^3/\mu\text{L}$ and females from 2.04 ± 0.18 to $15.43 \pm 0.52 \times 10^3/\mu\text{L}$. No necrotic lesions were identified in any dose group. Splenic congestion and extramedullary hematopoiesis were mild to moderate, remaining below 30–50% involvement without architectural disruption. These findings indicated that repeated oral exposure to *A. lappa* extract up to 450 mg/kg BW/day did not induce structural injury or suppress immune-cell profiles, supporting its preliminary safety for further toxicological and immunopharmacological investigation.

Keyword: *Arctium lappa* L., Burdock Root, Spleen, Lymphocytes, Histopathology, Wistar Rats

1. Introduction

Herbal medicines are increasingly utilized worldwide because they are accessible and affordable; in Indonesia, medicinal plants are commonly employed as traditional and complementary therapies (Faizal, Puspodewi, et al., 2024). Among promising candidates is burdock root an *Asteraceae* species widely cultivated in East Asia, including China and Japan, and traditionally used to alleviate infections, fever, dyspepsia, and chronic inflammatory disorders (Yosri et al., 2023). Contemporary studies report that burdock root contains fructooligosaccharides, phenolic acids, flavonoids, phenols, alkaloids, and saponins that exhibit anti-inflammatory, antioxidant, antimicrobial, and immunomodulatory activities (X. Zhang et al., 2019). These bioactive constituents may modulate the immune system by influencing effector cell populations such as lymphocytes. The immune system comprises innate (nonspecific) and adaptive (antigen-specific) arms that cooperate to protect the host (Faizal, Irmansa, et al., 2024). Lymphocytes – key leukocytes within adaptive immunity – mediate humoral

*) Corresponding Author (Imam Agus Faizal)
E-mail; imamdfaizal@universitasalirsyad.ac.id

responses via B-cell antibody production and cellular responses via T-cell cytotoxicity and helper functions (Prakoeswa et al., 2020). Much of this activity is orchestrated within secondary lymphoid organs. The spleen, the largest lymphoid organ, filters blood, generates antibodies, and supports T- and B-cell maturation; histological alterations such as white-pulp expansion, congestion, or extramedullary hematopoiesis can index immune activation or toxicity (Setiawan et al., 2021). The spleen is the largest lymphoid organ that plays an important role in blood filtration, antibody production, and maturation of T and B lymphocytes (Lutviana et al., 2022). Consequently, splenic histopathology, coupled with peripheral lymphocyte quantification, provides an informative readout for evaluating the safety and immunological impact of botanical products (Permana et al., 2023).

Despite increasing scientific interest in burdock (*Arctium lappa* L.), *in vivo* evidence that directly links standardized oral dosing with hematological and splenic outcomes remains limited. Previous studies have predominantly examined single-dose toxicity, localized wound-healing responses, or non-lymphoid parameters, resulting in an incomplete characterization of its systemic immunomodulatory safety. In addition, several reports lacked dose-response comparisons or employed non-oral administration routes, which reduces translational relevance considering that burdock is traditionally consumed orally. Oral exposure is essential to evaluate because its pharmacokinetics—including polysaccharide absorption, biotransformation, and first-pass hepatic metabolism—are route dependent (Liu et al., 2025). Establishing the safety profile of graded oral doses is therefore necessary before advancing burdock as a potential immunotherapeutic adjunct.

To address these gaps, this study investigates the effects of 28-day graded oral administration of burdock root extract on peripheral lymphocyte counts and spleen histopathology in Wistar rats. The design integrates a dose-response framework with predefined histopathological scoring to generate reproducible and interpretable data on splenic architecture and immune-cell dynamics. Both male and female rats were included to capture sex-related physiological variability, acknowledging that hormonal differences can influence lymphocyte counts, vascular responses, and extramedullary hematopoiesis. While hormonal fluctuations may introduce natural biological variation, their inclusion enhances ecological validity and aligns with current recommendations for sex-balanced preclinical research (Kosyreva et al., 2020). Through this approach, the study provides a more comprehensive and translational evaluation of the systemic safety and immunological profile of *Arctium lappa* L. under oral dosing conditions reflective of traditional human use.

2. Method

This experimental randomized controlled study evaluated the effects of graded oral doses of *Arctium lappa* L. root extract on lymphocyte counts and splenic histopathology in Wistar rats. The study was designed using a parallel-group dose-response framework. Eight healthy Wistar rats (*Rattus norvegicus*), aged 2–3 months and weighing 200–300 g, were used. The sample size ($n = 2$ per group) followed exploratory toxicology guidelines aimed at detecting overt histopathological changes while adhering to the principle of minimum animal use. Ethical approval was granted by the Ethics Committee of Al-Irsyad University Cilacap (No. 1150/234/03.3/2/3). Botanical authentication of *Arctium lappa* L. root was conducted by the Environmental Laboratory, Faculty of Biology, Universitas Jenderal Soedirman (Certificate No. B/314/UNI23.6.10/TA.00.01/2024). Animals were acclimatized for 7 days under controlled environmental conditions and were fasted for 12 hours prior to sampling.

Rats were randomly assigned to four groups: a negative control (vehicle) and three treatment groups receiving *Arctium lappa* L. extract at 150, 300, or 450 mg/kg body weight per day. The extract was prepared using maceration with 96% ethanol, following standardized herbal extraction procedures (Alifah et al., 2023). Each dose was administered once daily via oral gavage for 28 consecutive days. Oral administration was selected because burdock is traditionally consumed orally, and its polysaccharide absorption and biotransformation are strongly influenced by gastrointestinal and hepatic first-pass mechanisms (Yang et al., 2025).

Blood samples were collected through the retro-orbital sinus under light anesthesia. Lymphocyte counts were analyzed using an automated hematology analyzer calibrated for rodent parameters. Spleens were excised, fixed in 10% neutral buffered formalin, processed through graded alcohols and xylene, embedded in paraffin, sectioned at 3–5 μm , and stained with Hematoxylin-Eosin. Histological evaluation was performed at 100 $\times$ and 400 $\times$ magnification according to previously established laboratory protocols (Faizal et al., 2018). Histopathological changes were graded semiquantitatively based on established scoring criteria assessing congestion, necrosis, and extramedullary hematopoiesis as none, mild (<30%), moderate (30–60%), or marked (>60%). Observers were blinded to group allocation to reduce scoring bias (Wigati et al., 2024).

Data were presented as mean $\pm$ standard deviation. Lymphocyte count differences were analyzed descriptively due to the small sample size, supplemented by non-parametric tests such as Kruskal-Wallis with rank-based post-hoc evaluation where applicable. Effect sizes were calculated to enhance interpretability. Statistical analysis was conducted using SPSS version 26 with a significance level of $p < 0.05$.

3. Results and Discussion

Oral administration of *Arctium lappa* L. root extract at 150–450 mg/kg BW/day for 28 days resulted in no overt splenic injury. The splenic microarchitecture of treated rats preserved normal white-pulp and red-pulp delineation. Mild vascular congestion and low-grade extramedullary hematopoiesis (EMH) were observed across treatment groups, each remaining within the mild scoring range (<30%). No necrotic foci, disrupted pulp boundaries, or cytological degeneration were detected. Peripheral lymphocyte counts (mean $\pm$ SD) showed no statistically significant differences compared with controls ($p > 0.05$; $\eta^2 < 0.20$), indicating that the extract did not exert measurable immunosuppressive effects. All values remained within the physiological leukogram reference interval for Wistar rats. High-resolution HE photomicrographs (100 $\times$ and 400 $\times$) confirm these findings, demonstrating intact pulp structures with minimal EMH and congestion (Faizal et al., 2020; Rochmah et al., 2021; Wu, Weng et al., 2020; Yaghoubi et al., 2019).

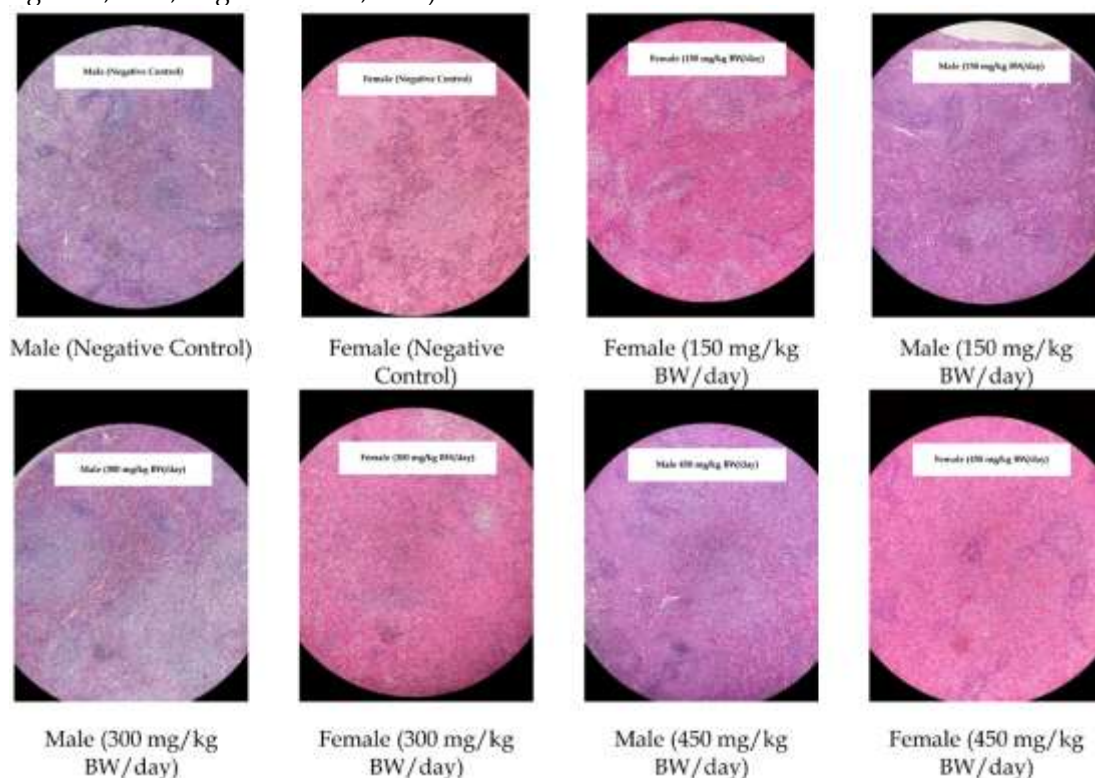


Figure 1. Representative HE-stained spleen sections of male and female Wistar rats from the negative

control and treatment groups receiving A. lappa L. extract at 150 mg/kg BW/day, 300 mg/kg BW/day, and 450 mg/kg BW/day (magnification 100 ×; three fields of view).

Figure 1 shows representative HE-stained spleen sections (100× magnification; three fields of view) from male and female Wistar rats across all treatment groups. The negative control animals demonstrated normal splenic architecture with clearly defined white-pulp and red-pulp regions and no evidence of necrosis. Mild vascular congestion was observed in both sexes. At 150 mg/kg BW/day, spleen morphology remained intact in male and female rats, with congestion classified as mild (<30%) based on semiquantitative scoring. No structural disruption of follicles, periarteriolar lymphoid sheaths, or red-pulp cords was detected. In the 300 mg/kg BW/day group, males exhibited mild congestion, while females showed mild-to-moderate congestion (30–60%). Low-grade extramedullary hematopoiesis (EMH), characterized by scattered megakaryocytes, was present but remained within the mild scoring range. No necrotic lesions were identified. At 450 mg/kg BW/day, spleens of both sexes preserved normal pulp organization. Congestion ranged from mild in males to mild-to-moderate in females. EMH was again limited to low-grade levels. Peripheral lymphocyte counts for all groups (mean ± SD) showed no statistically significant differences from controls ($p > 0.05$; $\eta^2 < 0.20$). All values remained within the expected physiological reference range for Wistar rats.

Overall, the preserved splenic microarchitecture across all treatment groups indicates that oral administration of *A. lappa* L. extract up to 450 mg/kg BW/day for 28 days does not induce cytotoxic damage. Mild congestion observed across groups is compatible with physiological hemodynamic variation and does not represent structural injury. Low-grade EMH likely reflects baseline hematopoietic activity and does not indicate pathological expansion. These findings are consistent with the reported antioxidant and immunomodulatory effects of burdock, which may contribute to maintaining splenic stability under metabolic stress (Yosri et al., 2023). The stable lymphocyte counts further support the absence of systemic immunosuppression. The study has limitations, including a small sample size ($n = 2/\text{group}$), which restricts statistical power and may under-detect subtle hematological changes. Biological variations—including sex-related hormonal effects—may influence congestion or EMH scoring, although standardized handling and blinded assessment reduce potential bias. Physiologically, the mild congestion observed in females at higher doses may reflect sex-specific vascular responses reported in rodent models (Anna M. Kosyreva et al., 2020). Future research should expand sample size, include cytokine and chemokine panels, perform flow-cytometric immune profiling, and assess oxidative-stress biomarkers to delineate dose-dependent immunological effects with greater precision (Liu et al., 2024).

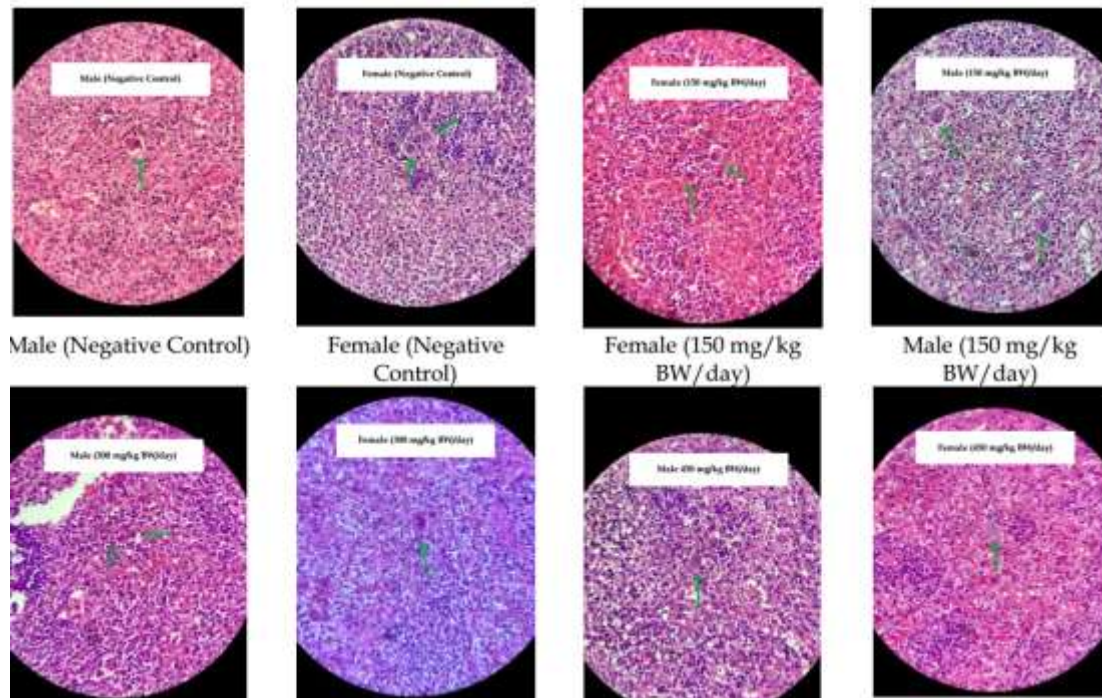


Figure 2. Hematopoiesis in the spleen of male and female Wistar rats in the negative control group and in groups receiving *A. lappa* L. extract at 150 mg/kg BW/day, 300 mg/kg BW/day, and 450 mg/kg BW/day (magnification 400 $\times$; five fields of view). Green arrow: megakaryocyte.

Figure 2 displays representative HE-stained spleen sections (400 $\times$ magnification; five fields of view) from male and female Wistar rats across all groups. In the negative control animals, isolated megakaryocytes were visible within the red pulp, consistent with physiological extramedullary hematopoiesis (EMH). In the 150 mg/kg BW/day group, EMH remained mild (<30%) in both sexes, with megakaryocytes appearing as solitary cells without clustering. Splenic architecture including white-pulp follicles, periarteriolar lymphoid sheaths, and red-pulp cords remained intact. Rats receiving 300 mg/kg BW/day demonstrated mild EMH in males and mild-to-moderate EMH (30 – 60%) in females, characterized by small groups of megakaryocytes within sinusoidal areas. No necrosis or architectural disruption was observed. At 450 mg/kg BW/day, EMH remained low-grade in both sexes. Megakaryocytes appeared individually or sparsely clustered without abnormal morphology. White- and red-pulp zoning remained preserved. Peripheral lymphocyte counts (mean $\pm$ SD) were not significantly different from controls ($p > 0.05$; small effect size), supporting the absence of treatment-related hematopoietic suppression.

The consistent presence of low-grade EMH across all groups suggests that burdock extract did not provoke pathological hematopoietic expansion or splenic injury at doses up to 450 mg/kg BW/day. The mild megakaryocytic activity observed in treated and control animals aligns with the physiological hematopoietic role of the rodent spleen. The preserved pulp organization and absence of necrosis reinforce the safety of the administered doses (Cenariu et al., 2021). The slightly higher EMH scoring in females at 300 mg/kg BW/day may reflect sex-related variation in splenic vascular responsiveness or hematopoietic sensitivity, a pattern commonly described in rodent immune physiology. Stable peripheral lymphocyte counts further indicate that the extract did not impair systemic immune cell production. Study limitations include the small sample size ($n = 2$ per group), which restricts inferential power and may reduce sensitivity to subtle histological changes. Biological variability—particularly sex-linked hormonal influences—may contribute to differences in EMH patterns (Gábris et al., 2023). Although blinding and standardized scoring procedures were applied, inter-individual variation cannot be fully eliminated. Nevertheless, the uniform histological profile across dose groups supports the conclusion that *A. lappa* extract is well tolerated under the tested conditions. Future work should integrate quantitative assays of hematopoietic markers, larger

cohorts, and multiparametric analyses such as cytokine profiling and flow-cytometric characterization of splenic cell subsets to refine mechanistic interpretation and dose-related immunological effects (Kuzmina et al., 2025).

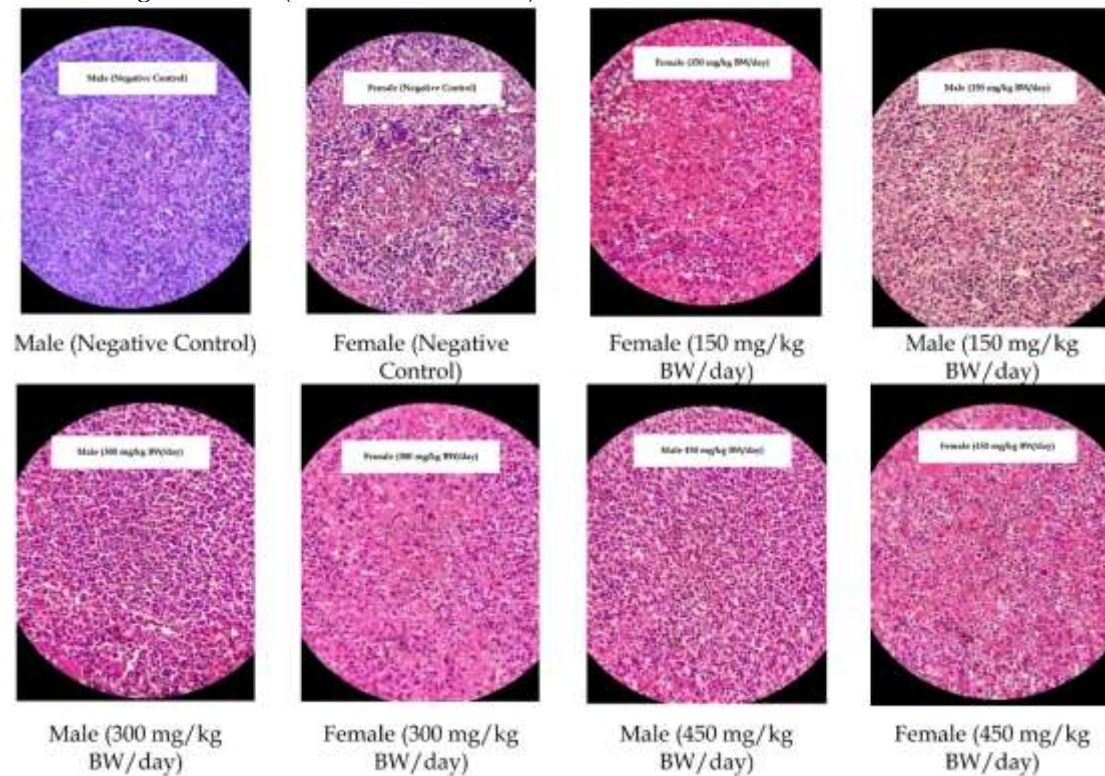


Figure 3. Assessment of necrosis in the spleen of male and female Wistar rats in the negative control group and in groups receiving *A. lappa* L. extract at 150 mg/kg BW/day, 300 mg/kg BW/day, and 450 mg/kg BW/day (magnification 400 $\times$; five fields of view). No necrotic lesions were detected in any treatment group.

Figure 3 presents representative HE-stained spleen sections (400 $\times$ magnification; five fields of view) from male and female Wistar rats across all treatment groups. In the negative control animals, the splenic parenchyma demonstrated intact white-pulp and red-pulp regions with no evidence of necrotic changes. Across treatment groups receiving *A. lappa* L. extract at 150, 300, and 450 mg/kg BW/day, no necrotic lesions were identified in either sex. The tissue morphology remained preserved, with normal cellular density and intact sinusoidal organization. No features of pyknosis, karyorrhexis, karyolysis, or cytoplasmic fragmentation were observed. Peripheral lymphocyte counts in all groups (mean $\pm$ SD) showed no significant differences compared with controls ($p > 0.05$; small effect size), and all values remained within physiological reference ranges, indicating an absence of dose-related cytotoxicity.

The uniform absence of necrotic lesions across all treatment groups indicates that 28-day oral administration of *A. lappa* L. extract does not induce structural or cellular damage to splenic tissue. Preservation of pulp architecture and the lack of nuclear or cytoplasmic degeneration confirm that the doses tested were well tolerated. Necrosis is a key indicator of toxic, ischemic, or inflammatory injury; therefore, its absence strongly supports the safety profile of the extract under these experimental conditions. Minor variations in tissue density or staining intensity observed between sexes were within normal physiological limits and did not resemble early degenerative changes. These findings align with the expected resilience of splenic tissue under non-stress conditions and further suggest that the extract does not exert pro-oxidative or pro-inflammatory effects at the administered doses (Guo et al., 2025).

The primary limitations of this study include the small sample size ($n = 2$ per group), which restricts statistical sensitivity, and potential biological variability related to sex-specific

microvascular or hormonal influences. Although randomization and blinded assessment reduce observer bias, subtle focal changes may remain undetected due to the limited sample number. Nevertheless, the consistent absence of necrosis across groups provides strong preliminary evidence of splenic safety. Future studies incorporating larger cohorts, quantitative assays of cell-death biomarkers, and extended exposure durations are warranted to more precisely characterize potential dose thresholds and longer-term safety (Cao et al., 2023)

Across treatment groups, splenic congestion reflected increased intravascular erythrocyte load within red-pulp sinusoids, consistent with transient hemodynamic redistribution rather than structural collapse. Congestion may arise from augmented inflow or impaired venous outflow; the relatively greater congestion observed in females is biologically plausible given sex-dependent endotoxin responsiveness—female rodents can display heightened sensitivity to lipopolysaccharide (LPS)–driven vascular changes—potentially amplifying microvascular pooling such congestion can accompany intensified immune surveillance and cell trafficking without implying toxicity (A. M. Kosyрева et al., 2014). Necrosis—defined by irreversible nuclear changes, including pyknosis, karyorrhexis, and karyolysis with chromatin loss—was not a dominant feature (I Wayan Mudiana et al., 2023). Mechanistically, necrosis follows severe energy failure (mitochondrial injury → ATP depletion) and ionic pump dysfunction (Na^+/K^+ ATPase), culminating in lysosomal rupture and hydrolytic autodigestion. Megakaryocytes—marrow-derived cells executing thrombopoiesis via terminal maturation (Zingariello et al., 2020) were present at low grade, indicating mild extramedullary hematopoiesis (EMH) rather than pathological expansion. The lack of a dose-related increase aligns with reports that *Arctium lappa* polysaccharides can temper platelet activation in a dose-dependent manner, thereby reducing thrombus propensity in vivo (Ruan et al., 2022). Together, the triad of reversible congestion, absent/covert necrosis, and only mild EMH supports our hypothesis that burdock extract maintains splenic microanatomy and immune homeostasis without eliciting injurious inflammation—consistent with its antioxidant and immunomodulatory profile.

Table 1. Baseline characteristics of Wistar rats

Group	n	Sex	Age (weeks)	Initial body weight (g)	Clinical condition
Negative control	2	Male/Female	9 ± 1	200–300	Healthy
150 mg/kg BW	2	Male/Female	9 ± 1	200–300	Healthy
300 mg/kg BW	2	Male/Female	9 ± 1	200–300	Healthy
450 mg/kg BW	2	Male/Female	9 ± 1	200–300	Healthy

Notes: Age is presented as mean ± SD; body weight reflects baseline (pre-treatment) measurements. "bw" = body weight.

Across groups, animals were age-matched (9 ± 1 weeks), within a narrow baseline weight range (200–300 g), and clinically healthy. This comparability minimizes confounding from maturational or cachectic effects that can alter splenic architecture, hematopoiesis, and circulating lymphocyte dynamics such baseline homogeneity strengthens internal validity for immunologic endpoints by reducing variance in thymic output, marrow cellularity, and stress-related leukocyte shifts. Although the per-group sample size is modest (n=2), the balanced sex composition and standardized acclimation/dosing windows support unbiased estimation of treatment effects on HE-based splenic histopathology and peripheral lymphocyte counts. Collectively, the baseline profile indicates that any observed differences are likely attributable to *Arctium lappa* exposure rather than pre-existing physiologic disparities, thereby enabling scientifically defensible inferences about safety and immunomodulatory potential (Ahmad et al., 2023)

Table 2. Interpretation of Splenic Tissue Alterations Based on Hematoxylin–Eosin (HE) Staining in Male Wistar Rats

Group	Congestion (100×)	Necrosis (400×)	Megakaryocytes (Extramedullary Hematopoiesis, 400×)
Negative Control	0	0	1.2
150 mg/kg BW/day	0	0	1.6
300 mg/kg BW/day	1	0	1.2
450 mg/kg BW/day	0	0	1.2

Notes: Scoring criteria: Score 0 = No lesion; Scores 1–2 = <30% involvement (mild alteration); Scores 2–3 = 30–50% involvement (moderate alteration); Scores 4–5 = >50% involvement (severe alteration); "bw" = body weight.

In male Wistar rats, splenic congestion scores ranged from 0 to 1 across all groups (Table 4.7). The negative control, 150 mg/kg BW/day, and 450 mg/kg BW/day groups exhibited no detectable congestion (score 0), whereas mild congestion (score 1) occurred only in the 300 mg/kg BW/day group. Necrosis was absent in all experimental groups (score 0). Megakaryocyte counts, reflecting extramedullary hematopoiesis (EMH), showed minimal variation, with values ranging from 1.2 to 1.6 across the treatment groups. Peripheral lymphocyte counts (mean $\pm$ SD) demonstrated no significant differences between groups ($p > 0.05$; small effect size), and all measurements remained within established physiological ranges for male Wistar rats. These findings support that no splenic cytotoxicity or hematopoietic suppression occurred at the tested doses.

The isolated mild congestion observed in the 300 mg/kg BW/day group without accompanying necrosis indicates a non-pathological microvascular response rather than a toxic effect. Mild congestion in the spleen is frequently reported as a normal physiological variation in rodent models and does not signify structural injury when pulp architecture remains intact (Chen et al., 2025). The uniform absence of necrosis across all dose groups further confirms that *A. lappa* L. extract did not induce cytotoxic or ischemic damage. Megakaryocyte counts remained within low physiological ranges, suggesting that splenic hematopoiesis was stable and not adversely affected by the extract. The stability of peripheral lymphocyte counts reinforces the interpretation that the extract does not impair systemic immune cell production in male rats (Guo et al., 2025b).

This study's limitations include the small sample size ($n = 2$ per group), which reduces statistical sensitivity and may obscure subtle histological changes. Biological variability – such as individual differences in microvascular tone or splenic perfusion – may also contribute to the mild congestion noted in one treatment group. Although randomization and blinded scoring were implemented to minimize bias, minor inter-individual variation cannot be completely excluded (Dervišević et al., 2025). Even with these limitations, the consistent absence of necrosis and stable EMH patterns across groups provide preliminary evidence that *A. lappa* L. extract is well tolerated in male rats at doses up to 450 mg/kg BW/day. Additional studies with larger cohorts and quantitative markers of endothelial stress or microvascular integrity may help clarify whether mild congestion reflects incidental variation or subtle physiological adjustment to repeated dosing (Lei et al., 2024).

Table 3. Interpretation of Splenic Tissue Alterations Based on Hematoxylin–Eosin (HE) Staining in Female Wistar Rats

Group	Congestion (100×)	Necrosis (400×)	Megakaryocytes (Extramedullary Hematopoiesis, 400×)
Negative Control	0	0	1.2
150 mg/kg BW/day	3.0	0	1.4
300 mg/kg BW/day	2.3	0	0.8
450 mg/kg BW/day	2.6	0	1.0

Notes: Scoring criteria: Score 0 = No lesion; Scores 1–2 = <30% involvement (mild alteration); Scores 2–3 = 30–50% involvement (moderate alteration); Scores 4–5 = >50% involvement (severe alteration); "bw" = body weight.

In female Wistar rats, splenic congestion scores ranged from 0 to 3.0 (Table 4.8). The negative control group showed no congestion (score 0). Marked congestion was observed in

the 150 mg/kg BW/day group (score 3.0), while the 300 mg/kg BW/day and 450 mg/kg BW/day groups demonstrated moderate congestion, with scores of 2.3 and 2.6, respectively. No necrotic lesions were detected in any of the experimental groups (score 0). Megakaryocyte counts, representing extramedullary hematopoiesis (EMH), varied between 0.8 and 1.4 but remained within the mild classification (<30%). Peripheral lymphocyte counts (mean $\pm$ SD) showed no statistically significant differences among groups ($p > 0.05$; $\eta^2 < 0.20$). All values remained within physiological limits for female Wistar rats.

The elevated congestion scores observed in female rats—particularly the marked congestion in the 150 mg/kg BW/day group—likely reflect sex-dependent microvascular variability rather than an adverse toxicological effect, as no necrosis was present in any group. Splenic congestion without associated cellular degeneration is typically considered a functional hemodynamic response and does not indicate structural damage when the parenchymal architecture remains preserved. This interpretation aligns with evidence showing that female rodents may exhibit distinct splenic vascular and immunophysiological responses under experimental conditions (Schrauf et al., 2023). Megakaryocyte counts across groups remained within the mild threshold, suggesting that splenic hematopoietic activity was maintained and not adversely affected by the extract. The stability of peripheral lymphocyte counts further supports the absence of systemic immunosuppression. Minor variations in congestion and EMH are consistent with normal biological variability and do not demonstrate a dose-related pattern (Menees et al., 2021).

This study is limited by the small sample size ($n = 2$ per group), which reduces statistical sensitivity and may underrepresent subtle histological differences. Sex-related physiological variation, including estrogen-linked modulation of splenic microcirculation, may influence congestion patterns. Although randomization and blinded scoring were applied, residual inter-animal differences cannot be excluded. Even with these limitations, the consistent absence of necrosis and the preservation of EMH patterns across all groups support the preliminary conclusion that *A. lappa* L. extract is well tolerated in female rats at doses up to 450 mg/kg BW/day. Further investigations incorporating quantitative endothelial markers, hormone-responsive vascular assays, and larger sample sizes may clarify whether the observed congestion represents normal physiological variation or subtle sex-specific reactivity to repeated oral exposure (J. Zhang, Bishir et al., 2025)

Table 4. Mean lymphocyte counts in Wistar rats ($10^3/\mu\text{L}$)

Group	Male	Female
Negative control	4.62	12.45
150 mg/kg BW/day	7.96	15.43
300 mg/kg BW/day	6.61	5.44
450 mg/kg BW/day	4.14	2.04

Notes: Values are means; units are $10^3/\mu\text{L}$. Reported reference interval: $0.86\text{--}5.66 \times 10^{23}/\mu\text{L}$ (as written in the source). This appears to be a typographical error and likely intended as $0.86\text{--}5.66 \times 10^3/\mu\text{L}$. "BW" = body weight.

After revising units, the reference interval should read $0.86\text{--}5.66 \times 10^3/\mu\text{L}$ (not $\times 10^{23}/\mu\text{L}$). Against this range, several groups show values above normal: males at 150 and 300 mg/kg BW/day (7.96 ; $6.61 \times 10^3/\mu\text{L}$) and females at control and 150 mg/kg BW/day (12.45 ; $15.43 \times 10^3/\mu\text{L}$). Others fall within range (male control 4.62 ; male 450 mg/kg 4.14 ; female 300 mg/kg 5.44) or low-normal (female 450 mg/kg BW/day 2.04). Thus, lymphocyte counts are not uniformly normal, and any claim of no difference requires formal statistics. These patterns suggest sex-by-dose variability rather than overt immunosuppression. Mild lymphocytosis at lower doses may reflect immunostimulatory signaling of antigenic priming, cytokine-mediated survival/trafficking, whereas normalization or decline at 450 mg/kg BW/day in females could indicate homeostatic counter-regulation or estrous-related shifts. Pre-analytical factors—retro-orbital sampling stress, diurnal timing, handling—can also influence leukograms. Given the small n (2/group), report mean $\pm$ SD and analyze with a two-way model (sex $\times$ dose) or a rank-based alternative; include effect sizes and CIs. Complement counts with flow cytometric subsets (CD3^+ , CD4^+ , CD8^+ , CD45RA/B -cell markers) and

cytokine panels to verify functional immunostimulation while corroborating the overall safety indicated by benign splenic histology (Kristono et al., 2020)

After correcting the reference unit to $0.86\text{--}5.66 \times 10^3/\mu\text{L}$, the leukograms show a sex-by-dose pattern rather than uniform normality: mild lymphocytosis appears at lower doses (male 150–300 mg/kg bw/day; female control–150 mg/kg bw/day), whereas values trend toward normalization or decline at the highest dose (female 450 mg/kg bw/day). Biologically, a non-monotonic profile is plausible for botanicals rich in polyphenols and polysaccharides: low-moderate exposure can enhance lymphocyte survival/trafficking via redox buffering and cytokine tone, while higher exposure engages counter-regulatory homeostasis (e.g., neuro-endocrine or endothelial set-points), without histologic injury. These dynamics are consistent with burdock's reported antioxidant and immunomodulatory activities, including effects of arctigenin, phenolics, and root polysaccharides (Shyam & Sabina, 2024).

The absence of splenic necrosis alongside only mild EMH and reversible congestion supports tolerability at 150–450 mg/kg bw/day. Polysaccharides from *A. lappa* can also temper platelet activation in vivo, aligning with low-grade EMH rather than pathological megakaryocytic expansion (Ruan et al., 2022)

Regarding sex differences, literature reports mixed endotoxin responsiveness between males and females depending on model, dose, and age; some data show higher female endotoxin and inflammatory indices after LPS, whereas other settings report male-biased responses. This variability supports interpreting our female congestion at higher doses as a microvascular adaptation rather than toxicity, while acknowledging the need for powered, sex-stratified analyses (Anna M. Kosyreva et al., 2020)

Methodologically, with $n=2/\text{group}$, claims of no difference require formal statistics (mean $\pm$ SD; two-way sex $\times$ dose model or rank-based alternative) and effect sizes/CI. To substantiate immunostimulation, pair counts with flow-cytometric subsets (e.g., CD3⁺, CD4⁺, CD8⁺, B-cell markers), cytokine panels, and oxidative-stress biomarkers. The benign splenic architecture coupled with selective lymphocytosis at lower doses is compatible with immunostimulatory potential and overall safety, meriting dose-response and time-course extensions (Shyam et al., 2024)

4. Conclusions and Suggestions

Taken together, the histopathological and hematological outcomes across all dose groups provide a coherent basis for interpreting the overall safety and immunological stability of *A. lappa* L. extract. At the highest dose (450 mg/kg BW/day), male rats showed no congestion or necrosis with mild EMH (1.2), while females exhibited moderate congestion (2.6) without necrosis and mild EMH (1.0). Lymphocyte counts remained within physiological or low-normal ranges, indicating preserved splenic integrity at maximal exposure. Graded oral *A. lappa* L. extract (150–450 mg/kg BW/day) preserved splenic architecture, caused only mild physiological alterations, and did not suppress lymphocyte levels, fulfilling the study objective. These findings demonstrate preliminary safety across the tested range and justify advancing to broader dose-response, cytokine-panel, and flow-cytometry studies to clarify its immunological mechanisms.

5. Acknowledgments

Faculty of Pharmacy, Science, and Technology, Al-Irsyad University Cilacap for the facilities provided during the research, we would like to express our gratitude to the KEPK of Jendral Soedirman University Purwokerto (UNSOED) Faculty of Medicine for its ethical permission, the Environmental Laboratory of the Faculty of Biology for its determination, and we would also like to express our gratitude to RA Hospital, Kartini Jepara for the preparation of histopathology preparations.

6. References

- Ahmad, N. (2023). In Vitro and In Vivo Characterization Methods for Evaluation of Modern Wound Dressings. *Pharmaceutics*, 15(1). doi:10.3390/pharmaceutics15010042
- Cao, L., Wei, L., Du, Q., Su, Y., Ye, S., & Liu, K. (2023). Spleen Toxicity of Organophosphorus Flame Retardant TDCPP in Mice and the Related Mechanisms. *Toxics*, 11(3). doi:10.3390/toxics11030231
- Cenariu, D., Iluta, S., Zimta, A. A., Petrushev, B., Qian, L., Dirzu, N., ... Zaharie, F. (2021, December 1). Extramedullary hematopoiesis of the liver and spleen. *Journal of Clinical Medicine*. MDPI. doi:10.3390/jcm10245831
- Chen, M., Zhang, Y., Zhao, Y., Cao, K., Niu, R., Guo, D., & Sun, Z. (2025). Complex immunotoxic effects of T-2 Toxin on the murine spleen and thymus: Oxidative damage, inflammasomes, apoptosis, and immunosuppression. *Ecotoxicology and Environmental Safety*, 289. doi:10.1016/j.ecoenv.2024.117476
- Dervišević, E., Čamdžić, N., Lazović, E., Salihbegović, A., Sessa, F., Spahović, H., & D'Errico, S. (2025). Histological and Immunohistochemical Evidence in Hypothermia-Related Death: An Experimental Study. *International Journal of Molecular Sciences*, 26(15). doi:10.3390/ijms26157578
- Faizal, I. A., Dwi, A., Widodo, W., & Nugraha, J. (2018). *The Effect of Macrophage Dose to Secretion Interleukin 6 (IL-6) on Model of Tuberculosis Granuloma In Vitro*. In *Proceedings of the 2nd International Conference Postgraduate School (ICPS)* (Vol. 6, pp. 306–311). Surabaya: Science and Technology Publications. doi:10.5220/0007541803060311
- Faizal, I. A., Irmansa, J., Pangesti, I., Oktaviani, F., Avivah, A. avivah L., Oktafiani, D. P., & Yuniasari, S. D. (2024). Assessing the Therapeutic Potential of *Arctium lappa* L. (Burdock Root) Ethanol Extract in Wound Healing on Male *Rattus norvegicus*. *Jurnal Teknologi Laboratorium*, 13(2), 8395. doi:10.29238/teknolabjournal.v13i2.417
- Faizal, I. A., Pangesti, I., & Purwati, R. (2020). The Decreased Levels of the Cooper (cu 2+) Ions in the Blood Clams (*Anadara granosa*) using the Pineapple (*Ananas comosus* (L.) merr.). *Jurnal Pharmaqueous: Jurnal Ilmiah Kefarmasian*, (02).
- Faizal, I. A., Puspodewi, D., Aji, A. P., Studi, P., Terapan, S., Laboratorium, T., ... Farmasi, F. (2024). Uji Toksisitas Subkronik Nanoemulsi Akar Burdock (*Arctium Lappa* L .) Berdasarkan Histopatologi pada Tikus Albino *Rattus noverticus* (strain Wistar). *Indonesian Journal of Innovation Multidiscipliner Research*, 2, 331–332.
- Gábris, F., Kiss, G., Szirmay, B., Szomor, Á., Berta, G., Jakus, Z., ... Balogh, P. (2023). Absence of Nkx2-3 induces ectopic lymphatic endothelial differentiation associated with impaired extramedullary stress hematopoiesis in the spleen. *Frontiers in Cell and Developmental Biology*, 11. doi:10.3389/fcell.2023.1170389
- Guo, Y., Li, Z., Xu, P., Guo, G., He, T., & Lai, Y. (2025a). Subchronic and Chronic Toxicity Assessment of Sublancin in Sprague-Dawley Rats. *Toxics*, 13(5). doi:10.3390/toxics13050413
- Guo, Y., Li, Z., Xu, P., Guo, G., He, T., & Lai, Y. (2025b). Subchronic and Chronic Toxicity Assessment of Sublancin in Sprague-Dawley Rats. *Toxics*, 13(5). doi:10.3390/toxics13050413
- I Wayan Mudiana, Sudisma, I. gusti N., Ni Luh Eka Setiasih, & I Wayan Sudira. (2023). Gambaran Histologi Hati Tikus Putih (*Rattus norvegicus*) yang Diberikan Ekstrak Bunga Kecubung (*Datura metel* L.) Sebagai Anestesi. *Acta VETERINARIA Indonesiana*, 11(2), 102–108. doi:10.29244/avi.11.2.102-108
- Kosyreva, A. M. (2014). The Sex Differences of Morphology and Immunology of SIRS of Newborn Wistar Rats. *International Scholarly Research Notices*, 2014, 1–7. doi:10.1155/2014/190749
- Kosyreva, Anna M., Dzhalilova, D. S., Makarova, O. V., Tsvetkov, I. S., Zolotova, N. A., Diatroptova, M. A., ... Mikhailova, L. P. (2020). Sex differences of inflammatory and immune response in pups of Wistar rats with SIRS. *Scientific Reports*, 10(1). doi:10.1038/s41598-020-72537-y
- Kristono, G. A., Holley, A. S., Hally, K. E., Brunton-O'Sullivan, M. M., Shi, B., Harding, S. A., & Larsen, P. D. (2020). An IL-6-IL-8 score derived from principal component analysis is

- predictive of adverse outcome in acute myocardial infarction. *Cytokine: X*, 2(4), 100037. doi:10.1016/j.cytox.2020.100037
- Kuzmina, M., Grusanovic, S., Brezina, J., Milosevic, M., Vanickova, K., Pavliuchenko, N., ... Alberich-Jorda, M. (2025). HSCs and Tregs cooperate to preserve extramedullary hematopoiesis under chronic inflammation. *BioRxiv*, 1–16. doi:10.1101/2025.02.05.636492
- Lei, L., Li, Y., Li, M., Xin, H., Tian, X., Zhang, Y., ... Cong, B. (2024). Pathological changes in the spleen of mice subjected to different time courses of restraint stress. *Scientific Reports*, 14(1). doi:10.1038/s41598-024-64475-w
- Liu, Y., Pan, W., Li, L., Gong, J., Dong, H., Zhang, B., ... Zheng, Z. (2024). Preparation and characterization of *Arctium lappa* L. polysaccharide-Zn(II) complex and evaluation of its immune activity. *Food Chemistry Advances*, 5. doi:10.1016/j.focha.2024.100784
- Lutviana, A., Adi, A. A. A. M., & Winaya, I. B. O. (2022). Viroterapi Virus Penyakit Tetelo pada Tikus Penderita Fibrosarkoma Tidak Berpengaruh terhadap Histopatologi Limpa. *Indonesia Medicus Veterinus*, 11(2), 147–158. doi:10.19087/imv.2022.11.2.147
- Menees, K. B., Earls, R. H., Chung, J., Jernigan, J., Filipov, N. M., Carpenter, J. M., & Lee, J. K. (2021). Sex- and age-dependent alterations of splenic immune cell profile and NK cell phenotypes and function in C57BL/6J mice. *Immunity and Ageing*, 18(1). doi:10.1186/s12979-021-00214-3
- Permana, D. A. S., Swandari, M. T. K., Faizal, I. A., Puspodewi, D., & Putri, A. I. A. (2023). The Effect of Doses of Red Shoot Leaf Extract (*Syzygium myrtifolium* Walp) on Decreased Cholesterol Total Levels in Male White Wistar Rats In Vivo. In *Atlantis Press (Springer nature)* (Vol. 225, pp. 262–273). doi:10.2991/978-94-6463-284-2_31
- Prakoeswa, F. R. (2020). Peranan Sel Limfosit Dalam Imunologi: Artikel Review. *Jurnal Sains Dan Kesehatan*, 2(4), 525–537. doi:10.25026/jsk.v2i4.212
- Rochmah, N. N., Elisa, I., Nugroho, Y. E., Faizal, I. A., & Utami, D. R. (2021). FORMULATION AND EVALUATION OF PHYSICAL PROPERTIES OF KATUK (*Sauropus androgynus* L Merr) LEAF EXTRACT TABLETS USING WET GRANULATION METHOD AS ANTIOXIDANT AND ANTIBACTERIAL. In *3rd Borobudur International Symposium 2021* (pp. 22–23). Magelang: Universitas Muhammadiyah Magelang Press. doi:10.36760/jpma.v3i2.337
- Ruan, Y., Ding, Y., Li, X., Zhang, C., Wang, M., Liu, M., ... Liu, Y. (2022a). Saccharides from *Arctium lappa* L. root reduce platelet activation and thrombus formation in a laser injury thrombosis mouse model. *Experimental and Therapeutic Medicine*, 23(5). doi:10.3892/etm.2022.11274
- Ruan, Y., Ding, Y., Li, X., Zhang, C., Wang, M., Liu, M., ... Liu, Y. (2022b). Saccharides from *Arctium lappa* L. root reduce platelet activation and thrombus formation in a laser injury thrombosis mouse model. *Experimental and Therapeutic Medicine*, 23(5), 344. doi:10.3892/etm.2022.11274
- Schrauf, S., Tomberger, Y., Nambulli, S., Duprex, W. P., Tschismarov, R., Tauber, E., & Ramsauer, K. (2023). Biodistribution and toxicology evaluation of a recombinant measles Schwarz-based Lassa vaccine in cynomolgus macaques. *Journal of Applied Toxicology*, 43(5), 719–733. doi:10.1002/jat.4421
- Setiawan, H., Wulandari, S. W., & Fitriyani, A. N. (2021). Potensi Imunomodulator Herbal Ekstrak Etanol Daun Pepaya Varietas Calina terhadap Struktur Jaringan Limpa Tikus Putih Galur Wistar. *Jurnal Veteriner*, 22(4), 531–539. doi:10.19087/jveteriner.2021.22.4.531
- Shyam, M., & Sabina, E. P. (2024, December 1). Harnessing the power of *Arctium lappa* root: a review of its pharmacological properties and therapeutic applications. *Natural Products and Bioprospecting*. Springer. doi:10.1007/s13659-024-00466-8
- Wigati, A. N., Faizal, I. A., & Puspodewi, D. (2024). Hepatotoxic Test Of Burdock Roots (*Arctium Lappa* L) On Histopathology Hepar Of Male White Rats (*Rattus Norvegicus*). *Jurnal Riset Kesehatan*, 13(2), 128–135. doi:10.31983/jrk.v13i2.11903
- Wu, K. C., Weng, H. K., Hsu, Y. S., Huang, P. J., & Wang, Y. K. (2020). Aqueous extract of *Arctium lappa* L. root (burdock) enhances chondrogenesis in human bone marrow-derived mesenchymal stem cells. *BMC Complementary Medicine and Therapies*, 20(1). doi:10.1186/s12906-020-03158-1

- Yaghoubi, M., Mousavi, Z., Rastegar, T., & Amin, G. (2019). Safety Assessment of Arctium lappa L. Fruit Extract in Female Wistar Rats: Acute and Repeated Oral Toxicity Studies. *Research Journal of Pharmacognosy (RJP)*, 6(2), 39–48. doi:10.22127/rjp.2019.84317
- Yang, M., Peng, G., Abudouwanli, A., Wang, S., Sun, Q., Zhao, W., ... Niyonsaba, F. (2025). Immunopharmacological potential of Arctium lappa L. in immune-mediated skin diseases: A critical review of experimental and clinical evidence. *Frontiers in Pharmacology*, 16. doi:10.3389/fphar.2025.1660352
- Yosri, N., Alsharif, S. M., Xiao, J., Musharraf, S. G., Zhao, C., Saeed, A., ... El-Seedi, H. R. (2023a). Arctium lappa (Burdock): Insights from ethnopharmacology potential, chemical constituents, clinical studies, pharmacological utility and nanomedicine. *Biomedicine & Pharmacotherapy*, 158(November 2022), 114104. doi:10.1016/j.biopha.2022.114104
- Yosri, N., Alsharif, S. M., Xiao, J., Musharraf, S. G., Zhao, C., Saeed, A., ... El-Seedi, H. R. (2023b). Arctium lappa (Burdock): Insights from ethnopharmacology potential, chemical constituents, clinical studies, pharmacological utility and nanomedicine. *Biomedicine and Pharmacotherapy*, 158. doi:10.1016/j.biopha.2022.114104
- Zhang, J., Bishir, M., Huang, W., & Chang, S. L. (2025). Sex dependent correlation of spleen atrophy and behavior deficits induced by binge treatment with ethanol in rodent models. *NeuroImmune Pharmacology and Therapeutics*, 4(1), 59–75. doi:10.1515/nipt-2024-0016
- Zhang, X., Liu, S., Lu, Y., Wang, J., & Chen, K. (2019). Immunomodulatory activity of a fructooligosaccharide isolated from burdock roots. *RSC Advances*, 9(20), 11092–11100. doi:10.1039/C8RA10091H
- Zingariello, M., Rosti, V., Vannucchi, A. M., Guglielmelli, P., Mazzarini, M., Barosi, G., ... Migliaccio, A. R. (2020). Shared and Distinctive Ultrastructural Abnormalities Expressed by Megakaryocytes in Bone Marrow and Spleen From Patients With Myelofibrosis. *Frontiers in Oncology*, 10(November), 1–12. doi:10.3389/fonc.2020.584541