International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

Drug-Induced Alterations in Splenic Structure and Function

SAIPRIYA S

Date of Submission: 20-05-2025 Date of Acceptance: 01-06-2025

ABSTRACT
The spleen is a critical organ involved in immune surveillance, hematopoiesis, and blood filtration, and it is increasingly recognized as a key target for drug-induced toxicity. This review provides a comprehensive overview of the anatomical and functional organization of the spleen and examines the mechanisms by which various classes of drugs induce structural and functional alterations. These mechanisms include direct cytotoxicity, oxidative stress, immunomodulation, and vascular remodeling, which collectively impact splenic architecture and systemic immune responses. Advances in imaging technologies, omics approaches, and novel in vitro models such as spleen-on-chip and organoids are enhancing the detection and mechanistic understanding of splenic toxicity. Integrating these emerging tools into preclinical safety assessments promises improved translational relevance and personalized therapeutic strategies. Continued research focused on the spleen is essential to bridge current knowledge gaps, develop spleen-protective therapies, and ensure safer pharmacological interventions. This review underscores the importance of spleen-focused pharmacological research in advancing drug safety and patient care.

KEYWORDS: spleen, drug-induced toxicity, immunomodulation, oxidative stress, spleen-on-chip, omics technologies, preclinical safety, pharmacology

I. INTRODUCTION

[1]The spleen, a vital secondary lymphoid organ located in the upper left quadrant of the abdomen, plays a pivotal role in maintaining immune surveillance, hematologic balance, and the removal of senescent erythrocytes. Despite being historically considered non-essential, modern biomedical research underscores its integral role in immunological function, erythrocyte turnover, and the pathophysiology of various systemic diseases, thereby making it a significant focus in pharmacological investigations.

[2]From a pharmacological perspective, the spleen is crucial not only as a site of drug distribution and metabolism but also as a target organ in immune-mediated and hematologic disorders. Its highly vascularized structure and immunologically active white pulp enable it to serve as a reservoir and mediator for various pharmacological agents, especially those targeting immune cells, reticuloendothelial function, and inflammatory mediators. [3]Moreover, splenic involvement is evident in conditions like splenomegaly, hypersplenism, hemolyticanemias, and infections—conditions for which pharmacological intervention often directly or indirectly affects spleen function.

The development of spleen-targeted therapies has gained momentum in recent years, with interest in natural products and phytochemicals demonstrating immunomodulatory, anti-inflammatory, and protective effects on splenic tissue. Pharmacological studies investigating these compounds provide insights into potential treatments for spleen-associated disorders while also aiding in understanding the systemic effects of drugs on lymphoid organs. Thus, evaluating the spleen's role in pharmacology can offer a comprehensive understanding of drug action in health and disease and pave the way for spleen-specific therapeutic strategies.

[4]In pharmacological research, the spleen is emerging as a significant organ for evaluating both **therapeutic efficacy** and **toxicological outcomes**. Due to its immunological and hematological functions, the spleen actively participates in drug disposition, immune modulation, and systemic inflammatory responses. For instance, immunomodulatory drugs, such as corticosteroids, monoclonal antibodies, and cytokine inhibitors, exert part of their action by influencing splenic lymphocyte populations and macrophage activity. Likewise, the spleen is a major site for antigen presentation, making it crucial in the pharmacodynamics of vaccines and immune-targeting agents.

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

[5]Moreover, the spleen can serve as a **biomarker organ** in preclinical toxicology studies. Changes in spleen weight, architecture, and cellularity are often included in safety pharmacology protocols to assess immunotoxic potential. For example, splenic atrophy or lymphoid depletion may indicate immunosuppression, while hyperplasia could suggest overstimulation or autoimmunity induced by chronic drug exposure.

[6]From a pharmacokinetic perspective, the spleen's mononuclear phagocyte system (MPS) plays a role in the clearance of particulate drug delivery systems, such as liposomes and nanoparticles. Understanding splenic uptake and sequestration is thus critical when designing drug carriers or formulating biologics.

[7]Furthermore, experimental models of **drug-induced toxicity**, such as those using doxorubicin, cyclophosphamide, or anti-tubercular drugs, have shown the spleen to be a secondary site of oxidative stress, apoptosis, and immunosuppression, highlighting the need for spleen-protective strategies in pharmacological interventions.These insights collectively position the spleen as not only a target but also a modulator of drug effects. Therefore, its inclusion in pharmacological and toxicological assessments is essential for a comprehensive understanding of drug action and safety.

ANATOMICAL AND FUNCTIONAL OVERVIEW OF THE SPLEEN

STRUCTURAL ORGANIZATION

[8]The spleen is encased in a dense **fibrous capsule**, from which trabeculae extend into the parenchyma, dividing it into compartments. Internally, the spleen is composed of two distinct regions:

White Pulp:

[9]The white pulp is lymphoid tissue organized around central arterioles. It consists of periarteriolar lymphoid sheaths (PALS), mainly populated by T lymphocytes, and lymphoid follicles rich in B cells. This region is analogous to lymph nodes and is responsible for initiating adaptive immune responses to antigens present in the blood.

Red Pulp:

[10]Surrounding the white pulp is the red pulp, which constitutes the majority of the splenic mass. It consists of blood-filled sinusoids and splenic cords (cords of Billroth). The red pulp is primarily involved in filtering blood, removing senescent or damaged erythrocytes, and recycling iron.

Marginal Zone:

[11]The marginal zone lies at the interface between the white and red pulp. It contains specialized macrophages, dendritic cells, and marginal zone B cells. This region plays a key role in trapping and processing blood-borne antigens, serving as a critical site for the early stages of immune response.

Capsule and Trabeculae:

The capsule provides structural support and protection to the spleen. Its trabeculae contain smooth muscle and collagen fibers, contributing to the organ's ability to contract and modulate blood flow during physiological and pathological states.

FUNCTIONAL ROLES

Immune Surveillance:

[12]The spleen is a major site for immune activation against blood-borne pathogens. Antigens are captured by antigen-presenting cells in the marginal zone and presented to T and Blymphocytes in the white pulp, triggering adaptive immune responses. The spleen is especially important in mounting responses to encapsulated bacteria such as *Streptococcus pneumoniae*.

Hematopoiesis:

[13]During fetal development, the spleen functions as a site of hematopoiesis. Although this function diminishes after birth, it can be reactivated under pathological conditions such as severe anemia or bone marrow failure (extramedullaryhematopoiesis.

Blood Filtration and Erythrocyte Clearance:

The red pulp filters circulating blood and removes senescent, damaged, or opsonized red blood cells and platelets. Macrophages within the splenic cords engulf these cells and recycle their components, particularly iron from haemoglobin.

Reservoir of Blood Cells:

[14]The spleen also serves as a reservoir for monocytes and platelets, which can be rapidly mobilized in response to injury or inflammation.Understanding the spleen's anatomical features and physiological roles provides a solid foundation for evaluating the impact of pharmacological agents on splenic structure and function. It also underscores the importance of including splenic endpoints in preclinical and clinical drug evaluation

MECHANISMS OF DRUG-INDUCED ALTERATIONS IN THE SPLEEN

The spleen, as a dynamic organ involved in both hematological and immunological functions, is particularly sensitive to the adverse effects of xenobiotics and pharmacological agents. Several classes of drugs have been reported to induce

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

pathological changes in the spleen through mechanisms that include direct cellular injury, oxidative stress, immune modulation, and stromal remodeling. These changes are of clinical importance, as they may compromise immune competence and contribute to systemic toxicity.

1. DIRECT CYTOTOXICITY

Direct cytotoxic effects occur when pharmacological agents interact with cellular components of the spleen, leading to apoptosis, necrosis, or depletion of specific cell types. Antineoplastic agents like **doxorubicin**, **cyclophosphamide**, and **busulfan** have been widely documented to cause white pulp atrophy due to their impact on rapidly dividing lymphocytes. The PALS and germinal centers, rich in T and B lymphocytes respectively, are particularly vulnerable.

In addition, **antiretroviral drugs** such as zidovudine and **immunosuppressants** like cyclosporine have shown lymphoid depletion and sinusoidal congestion in experimental models. These changes are often dose- and duration-dependent and may lead to functional immunodeficiency if sustained. Moreover, antibiotics like chloramphenicol, when used chronically, may lead to hypoplasia or aplasia of splenic tissue (Olson et al., 2000).

The mechanisms of cytotoxicity often involve interference with nucleic acid synthesis, protein production, or mitochondrial respiration, ultimately leading to cell death and architectural disintegration.

2. OXIDATIVE STRESS AND INFLAMMATION

A significant number of drugs induce **oxidative stress** in splenic tissues through the generation of reactive oxygen species (ROS) that overwhelm the antioxidant defense systems. **Cisplatin**, **acetaminophen**, and **isoniazid** are known to produce ROS, which damage cellular lipids, proteins, and DNA in splenic macrophages and lymphocytes (Hamza et al., 2018; Abdel-Daim et al., 2019).

This oxidative damage often triggers the activation of redox-sensitive transcription factors like NF-κB and AP-1, leading to the upregulation of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β. These mediators recruit and activate splenic immune cells, exacerbating tissue injury and potentially resulting in **splenomegaly**, congestion, and infiltration of neutrophils and monocytes.

Oxidative stress may also impair the function of the reticuloendothelial system, reducing the spleen's ability to clear damaged or infected erythrocytes, thereby increasing susceptibility to infections and contributing to systemic inflammation.

3. IMMUNOMODULATION AND LYMPHOID ATROPHY

Drugs that modulate immune activity—either suppressively or stimulatory—can significantly impact splenic lymphoid architecture. **Corticosteroids** like dexamethasone and prednisolone exert potent immunosuppressive effects by inducing apoptosis in thymocytes and peripheral T cells, leading to marked depletion in the white pulp regions of the spleen (Kuper et al., 2000).

Similarly, **cytotoxic drugs** used in chemotherapy regimens and **biological therapies** such as monoclonal antibodies (e.g., rituximab) can deplete specific lymphocyte populations and lead to follicular atrophy or marginal zone disruption. Chronic administration may also reduce germinal center formation and impair antibody production.

Conversely, **immune checkpoint inhibitors** and some **cytokine therapies** (e.g., IL-2 or IFN-γ) can induce **hyperactivation** of immune cells, resulting in lymphoid hyperplasia, increased mitotic figures in follicles, and potential autoimmune phenomena.

Long-term alterations in lymphocyte dynamics not only compromise adaptive immunity but also disrupt splenic homeostasis, predisposing the host to infections and autoimmune sequelae.

4. VASCULAR AND STROMAL REMODELING

Beyond lymphoid effects, many drugs can influence the vascular and connective tissue architecture of the spleen. **Chemotherapeutic agents**, **radiation**, and **anti-angiogenic drugs** like bevacizumab can cause endothelial injury, microvascular thrombosis, and hemorrhage in the red pulp, leading to **congestion**, **fibrosis**, and **capsular thickening** (Cesta, 2006; Mebius& Kraal, 2005).

These vascular injuries may impair blood filtration and lead to splenic infarction in severe cases. Drugs such as hydralazine or methyldopa, known for their vascular effects, may also lead to changes in splenic sinusoid permeability and blood pooling, resulting in transient splenomegaly or hypoxia-induced necrosis.

Additionally, **stromal remodeling** may occur due to activation of fibroblasts and extracellular matrix deposition. Fibrosis, observed in chronic drug

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

exposure or in association with systemic inflammation, can reduce splenic elasticity and limit its ability to contract and expel stored blood cells during physiological demand.

These structural alterations may also reduce the spleen's capacity to act as a reservoir for platelets and monocytes, influencing hemostasis and wound healing.

Drug-induced alterations in the spleen arise from multifaceted mechanisms involving direct cellular injury, oxidative insult, immunomodulation, and vascular remodeling. These changes can affect both structure and function of the spleen, with clinical consequences ranging from impaired immune surveillance to systemic hematological disturbances. Recognizing these mechanisms is essential in toxicological evaluation and may guide safer therapeutic strategies.

CLASSES OF DRUGS AND THEIR EFFECTS ON SPLENIC STRUCTURE

The spleen, as a critical immune and hematopoietic organ, is sensitive to various pharmacological agents. Different drug classes induce characteristic structural and functional changes in the spleen, reflecting their mechanisms of action, therapeutic targets, and toxicities. Understanding these effects is vital for predicting adverse outcomes, guiding clinical management, and designing protective interventions.

CHEMOTHERAPEUTIC AGENTS

Chemotherapeutic agents, while essential for cancer treatment, are well known for their cytotoxicity toward non-target tissues, including the spleen. The spleen's high cellular turnover and rich lymphoid content make it particularly susceptible to chemotherapy-induced damage.

Cyclophosphamide

Cyclophosphamide is an alkylating agent that requires hepatic activation to form active metabolites causing DNA cross-linking, inhibiting cell division (Nair et al., 2014). In the spleen, this results in:

- **Lymphoid depletion:** Significant reduction of lymphocytes in white pulp, leading to decreased germinal centers and impaired adaptive immunity (Elmore, 2007).
- **Vascular congestion:** Damage to sinusoidal endothelial cells causes blood pooling in the red pulp.
- **Fibrosis:** Repeated or high-dose exposure leads to stromal fibrosis, impairing spleen elasticity and function (Sharma et al., 2018).

Animal models show reduced splenic weight and cellularity after cyclophosphamide treatment, correlating with systemic immunosuppression (Al-Muzafar& Amin, 2017).

Doxorubicin

Doxorubicin, an anthracycline antibiotic, intercalates DNA and produces reactive oxygen species (ROS) through redox cycling, causing oxidative damage (Nair et al., 2014). Its splenic effects include:

- **Endothelial injury:** Sinusoidal capillaries are damaged, leading to hemorrhage and congestion in the red pulp.
- **Apoptosis induction:** Activation of caspase pathways results in lymphocyte and stromal cell death (Rauniyar et al., 2020).
- **Dose-dependent damage:** Higher cumulative doses cause profound architectural disruption and fibrosis.

Antioxidant co-treatment, such as with vitamin E or polyphenols, shows promise in attenuating these effects (Abdel-Daim et al., 2019).

4.1.3 Methotrexate and Others

Methotrexate inhibits folate metabolism, suppressing DNA synthesis in proliferative cells, including splenic lymphocytes (Cesta, 2006). Prolonged use causes:

- **White pulp atrophy:** Loss of lymphocytes and decreased immunoglobulin production.
- **Immunosuppression:** Heightened risk of infection due to impaired antigen response.

Other chemotherapeutics (e.g., cisplatin, paclitaxel) also cause variable splenic toxicities mainly through oxidative stress and apoptosis induction.

IMMUNOMODULATORS AND BIOLOGICS

Immunomodulatory drugs and biologics elicit diverse responses in the spleen depending on their mechanism, ranging from immune activation to suppression.

VACCINES

Vaccines stimulate antigen-presenting cells and lymphocytes in the spleen to mount adaptive immune responses.

White pulp hyperplasia: Expansion of B- and T-cell zones with increased germinal center formation (Chen &Mellman, 2017).

Follicular activation: Enhanced proliferation of plasma cells producing antigen-specific antibodies.

These changes are typically transient and reflect a healthy immune response.

MONOCLONAL ANTIBODIES

Monoclonal antibodies target specific immune cells or receptors, altering splenic lymphoid populations.

Rituximab: Anti-CD20 antibody depletes B cells, leading to white pulp lymphoid follicle involution (Kroemer et al., 2015).

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

Impact: May cause temporary hypogammaglobulinemia and reduced splenic immune surveillance.

Other biologics (e.g., anti-TNF agents) modulate inflammatory pathways, with variable effects on splenic cellularity.

IMMUNE CHECKPOINT INHIBITORS

These drugs unleash immune responses by blocking inhibitory signals but can also cause autoimmune reactions affecting the spleen.

- **Lymphoid hyperplasia or atrophy:** Depending on patient response, either expansion or depletion of lymphocytes may occur.
- **Autoimmune splenitis:** Rarely, immune-mediated inflammation and tissue damage are observed.

ANTI-INFLAMMATORY AND ANTIOXIDANT AGENTS

Drugs that reduce inflammation and oxidative stress can protect spleen architecture and function.

- **NSAIDs:** By inhibiting cyclooxygenase enzymes, NSAIDs decrease prostaglandin-mediated inflammation in the spleen.
- **Polyphenols (e.g., quercetin, resveratrol):** Scavenge free radicals and inhibit NF-κBsignaling, reducing inflammatory cytokine release.
- **Histological effects:** Preservation of lymphoid follicles, decreased congestion, and reduced stromal fibrosis.

These agents are often used as adjuncts to protect the spleen during chemotherapeutic or other toxic exposures.

HEMATOPOIETIC AND HEPATOPROTECTIVE DRUGS

Though acting systemically, these drugs impact spleen health indirectly.

- **Erythropoietin (EPO):** Stimulates erythroid progenitors in spleen during anemia, causing extramedullaryhematopoiesis (Arafa et al., 2020).
- **G-CSF:** Mobilizes hematopoietic stem cells and can expand splenic myeloid populations.
- **Silymarin:**Hepatoprotective flavonoid reducing liver-derived toxins, thereby lessening splenic injury (Polyak et al., 2010).

PLANT-BASED AND NATURAL COMPOUNDS

Natural products have shown promise in preventing or reversing drug-induced splenic damage.

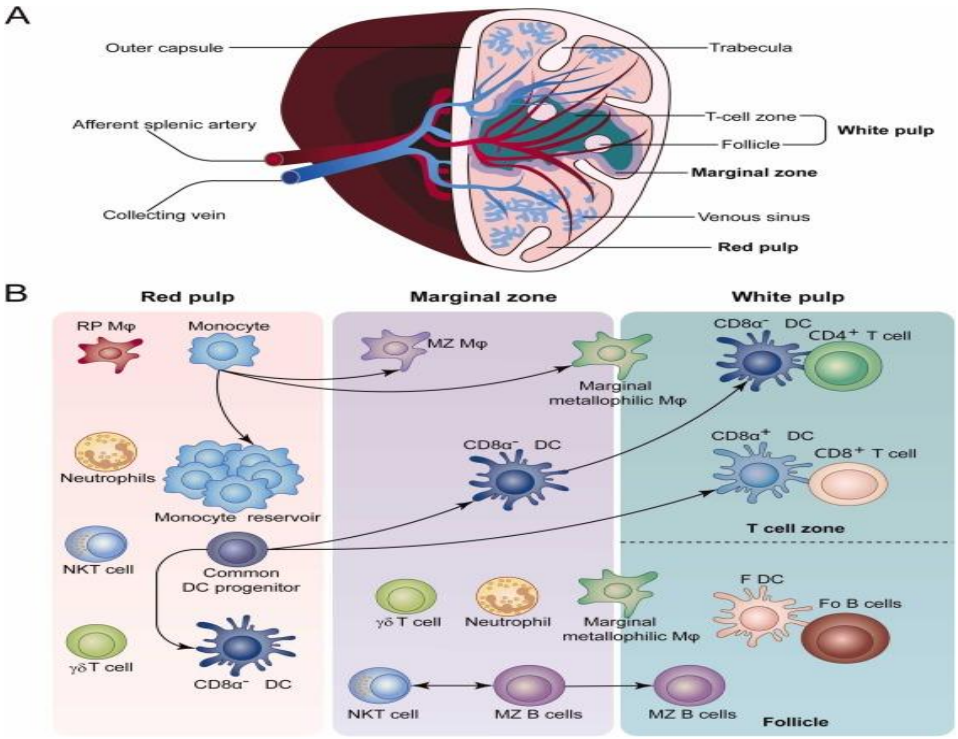
- *Ipomoea digitata* contains flavonoids and saponins with antioxidant and immunomodulatory activity, promoting restoration of normal splenic architecture (Rauniyar et al., 2020).
- *Nymphaea nouchali* exhibits anti-inflammatory properties in rodent spleen injury models, improving lymphoid follicle integrity (Sharma et al., 2022).
- *Tinosporacordifolia* modulates immune function and reduces oxidative stress in spleen during chemotherapy (Sharma et al., 2018).

These herbal agents represent promising adjuncts in mitigating chemotherapy and other drug toxicities.

Table 1: Summary of Drug Classes and Their Effects on Spleen

Drug Class	Examples	Mechanisms	Splenic Changes	References
Chemotherapeutics	Cyclophosphamide, Doxorubicin	DNA damage, ROS generation	Lymphoid depletion, congestion	Elmore (2007), Nair et al. (2014)
Immunomodulators/ Biologics	Vaccines, Rituximab	Immune activation/depletion	Follicular hyperplasia/atrophy	Chen &Mellman (2017), Kroemer et al. (2015)
Anti-inflammatory agents	NSAIDs, Polyphenols	COX inhibition, antioxidant	Preservation of architecture	Abdel-Daim et al. (2019), Sharma et al. (2018)
Hematopoietic drugs	Erythropoietin, G-CSF	Stimulate hematopoiesis	Extramedullaryhematopoiesis	Arafa et al. (2020)
Natural compounds	*Ipomoea digitata*, *Tinosporacordifolia*	Antioxidant, immunomodulatory	Restoration of splenic follicles	Rauniyar et al. (2020), Sharma et al. (2018)

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494



EXPERIMENTAL MODELS AND ASSESSMENT TECHNIQUES

Studying drug-induced splenic changes necessitates the use of reliable experimental models and assessment methods that enable detailed evaluation of structural and functional alterations. Rodent models, particularly rats and mice, are extensively utilized due to their physiological similarity to humans and well-characterized immune systems. This section elaborates on animal models of splenic toxicity and protection, followed by histological, immune histochemical, morphometric, and biochemical techniques commonly employed to assess splenic injury and recovery.

ANIMAL MODELS FOR SPLENIC TOXICITY OR PROTECTION

Rodent models are the mainstay for evaluating splenic toxicity induced by chemotherapeutic agents, immunomodulators, and other pharmacological compounds. The **doxorubicin-induced splenic toxicity model** is widely used; administration of doxorubicin at cumulative doses (e.g., 15 mg/kg intraperitoneally) causes oxidative stress, apoptosis, and histopathological damage within splenic tissue (Rauniyar et al., 2020). Similarly, **cyclophosphamide models** involve dosing in the range of 100–200 mg/kg, leading to lymphoid depletion and vascular congestion (Al-Muzafar& Amin, 2017). These models recapitulate immunosuppressive effects observed clinically and provide a platform for evaluating potential protective agents.

Protective models typically involve co-administration or pretreatment with antioxidant or herbal extracts. For instance, co-treatment with *Ipomoea digitata* tuber extract has been shown to ameliorate doxorubicin-induced splenic damage through antioxidant and anti-apoptotic mechanisms (Sharma et al., 2018). Likewise, antioxidant supplementation with compounds such as vitamin E or quercetin can restore splenic architecture by mitigating reactive oxygen species (ROS) formation (Abdel-Daim et al., 2019). Additionally, immunomodulatory drug models employing vaccines or monoclonal antibodies enable assessment of splenic white pulp hyperplasia or lymphoid depletion (Chen &Mellman, 2017). Genetic knockout models further facilitate exploration of molecular pathways underlying splenic injury and repair.

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Histological analysis remains the cornerstone for assessing splenic morphology following drug exposure. Spleen specimens are fixed, processed, and sectioned for staining. Routine

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

hematoxylin and eosin (H&E) staining provides insight into overall tissue architecture, highlighting lymphoid depletion, vascular congestion, and fibrosis (Kroemer et al., 2015). Special stains such as **Masson's trichrome** are employed to detect collagen deposition indicative of fibrosis, while **Prussian blue** stain identifies iron deposits related to hemorrhage.

Immunohistochemistry (IHC) allows for cell-specific and molecular-level investigation. Markers such as **CD3** and **CD20** identify T and B lymphocytes respectively, enabling evaluation of lymphoid population changes. **Ki-67** staining detects proliferating cells, and **caspase-3** serves as an apoptosis indicator. Vascular integrity can be assessed using endothelial markers like **CD31** or **von Willebrand factor** (Kroemer et al., 2015). IHC thus provides quantitative and qualitative data on immune cell status and tissue damage.

MORPHOMETRIC AND BIOCHEMICAL ANALYSES

Morphometric analysis quantifies splenic structural changes objectively. Digital image analysis software, such as ImageJ, is utilized to measure parameters including white pulp area, follicle count, and red pulp vascular density. Cell counts for lymphocytes, macrophages, or apoptotic cells further refine histological findings.

Biochemical assays complement morphological studies by assessing oxidative stress and inflammatory status. Markers such as **malondialdehyde (MDA)** indicate lipid peroxidation, while enzymatic antioxidants including **superoxide dismutase (SOD)**, catalase, and **glutathione (GSH)** reflect the tissue's defense capacity (Abdel-Daim et al., 2019). Cytokine profiling through ELISA or multiplex assays measures pro-inflammatory (TNF-α, IL-6) and anti-inflammatory (IL-10) mediators. Additionally, enzymatic activities like acid and alkaline phosphatase offer insight into macrophage and lysosomal function. Protein expression studies via Western blot or RT-PCR analyze apoptotic and inflammatory pathway components such as Bax, Bcl-2, and NF-κB.

A recent study by Rauniyar et al. (2020) exemplifies the integration of these methods: doxorubicin administration resulted in lymphoid depletion and congestion on H&E staining, elevated caspase-3 expression via IHC, and increased MDA with decreased antioxidant enzymes in biochemical assays. Treatment with *Ipomoea digitata* reversed these changes, highlighting the utility of combined assessment techniques.

Summary of Experimental Models and Assessment Techniques for Studying Drug-Induced Splenic Changes

Technique Category	Method/Parameter	Application Example
Animal Models	Doxorubicin (15 mg/kg), Cyclophosphamide (100–200 mg/kg)	Induction of splenic toxicity
	Herbal extract co-treatment	Assessment of splenic protection
Histology	Hematoxylin and Eosin, Masson's Trichrome, Prussian Blue	Evaluation of lymphoid depletion, fibrosis
Immunohistochemistry	CD3, CD20, Ki-67, Caspase-3, CD31	Immune cell profiling, apoptosis, vascular integrity
Morphometry	White pulp area, follicle number, cell counts	Quantitative structural analysis
Biochemical Assays	MDA, SOD, catalase, GSH, cytokines (TNF-α, IL-6, IL-10)	Oxidative stress and inflammation markers

CLINICAL IMPLICATIONS OF SPLENIC CHANGES

The spleen is a vital organ involved in immune regulation, hematological homeostasis, and filtration of blood-borne pathogens and cellular debris. Drug-induced alterations in splenic structure and function can significantly impact patient outcomes, influencing drug safety profiles, immune competence, and susceptibility to infections and autoimmune diseases. Understanding these clinical implications is essential for healthcare providers to optimize therapeutic strategies and minimize adverse effects.

DIAGNOSTIC RELEVANCE IN DRUG SAFETY

The spleen is a key organ assessed during preclinical toxicological studies due to its sensitivity to xenobiotic-induced injury. Histopathological changes such as lymphoid depletion, red pulp congestion, hemorrhage, fibrosis, or follicular hyperplasia often serve as early indicators of

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

systemic toxicity (Patel et al., 2019). These morphological alterations provide insight into the immunotoxic potential of new pharmacological agents, guiding dose selection and safety margins.

In clinical settings, splenic changes may be detected incidentally during imaging or autopsy, raising concerns about drug-related adverse effects. For example, MRI and ultrasonography can detect splenomegaly, infarcts, or fibrosis secondary to drug-induced inflammation or vascular injury (Fayad et al., 2018). Additionally, splenic biomarkers, such as circulating immune cell counts and serum cytokines, may correlate with tissue changes, offering non-invasive monitoring tools for immunotoxicity.

Recognition of drug-induced splenic injury is critical, particularly with chemotherapeutic agents like doxorubicin and cyclophosphamide, which cause dose-dependent toxicity. Early identification enables clinicians to adjust therapy, incorporate protective agents, or implement supportive care to mitigate adverse effects (Rauniyar et al., 2020). Regulatory agencies now emphasize spleen evaluation as part of comprehensive safety pharmacology assessment.

IMPACTS ON IMMUNITY, INFECTION RISK, AND AUTOIMMUNITY

The spleen is indispensable for both innate and adaptive immunity. Its white pulp functions as a site for lymphocyte activation and antibody production, while the red pulp filters blood and removes defective erythrocytes and pathogens. Drug-induced structural changes can impair these functions, leading to clinical consequences.

Immunosuppression and Infection Risk

Lymphoid depletion or follicular atrophy due to cytotoxic drugs reduces B and T cell populations, weakening the host's ability to mount effective immune responses (Mebius& Kraal, 2005). This immunosuppression heightens vulnerability to opportunistic infections, particularly by encapsulated bacteria such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. The risk of severe, sometimes fatal infections increases markedly in patients with functional or anatomical asplenia (Davies et al., 2011).

Moreover, splenic macrophages play a critical role in clearing circulating pathogens. Their depletion or dysfunction due to drug toxicity compromises innate immunity and delays pathogen clearance, prolonging infections and increasing systemic inflammation. This is especially significant in cancer patients receiving chemotherapy, who often develop neutropenia and splenic dysfunction concurrently.

Autoimmunity and Immune Dysregulation

Paradoxically, some pharmacological agents, especially immunomodulators and biologics, may induce splenic hyperplasia and follicular activation, reflecting immune system overstimulation (Rheumatoid Arthritis Study Group, 2017). This heightened immune activity can lead to aberrant immune responses and the development or exacerbation of autoimmune conditions such as systemic lupus erythematosus, rheumatoid arthritis, and autoimmune hemolyticanemia.

Certain drugs may unbalance splenic regulatory T cell populations or alter cytokine profiles, promoting chronic inflammation and autoantibody production. Monitoring splenic changes in patients receiving such therapies is essential to prevent immune-mediated adverse events.

CLINICAL MANAGEMENT CONSIDERATIONS

Patients with drug-induced splenic impairment require careful clinical management. Preventive measures include vaccination against encapsulated bacteria and prophylactic antibiotics in cases of functional asplenia or severe immunosuppression (Davies et al., 2011). Periodic monitoring of splenic size and function through imaging and laboratory markers is recommended during treatment with high-risk drugs.

[15]Additionally, the use of spleen-protective agents such as antioxidants, herbal extracts, or immunomodulatory supplements may improve outcomes by preserving splenic architecture and immune function. For example, preclinical studies indicate that *Ipomoea digitata* and other plant-derived compounds can restore splenic cellularity and reduce oxidative damage induced by chemotherapy.

Understanding the clinical implications of splenic changes also aids in patient education, emphasizing infection risk reduction, prompt recognition of febrile illness, and adherence to vaccination schedules. Integrating splenic assessment into routine clinical practice will enhance patient safety and therapeutic efficacy.

CHALLENGES, LIMITATIONS, AND KNOWLEDGE GAPS

Despite significant advances in understanding drug-induced splenic changes, several challenges and limitations remain that hinder comprehensive

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

evaluation and clinical translation. Addressing these gaps is crucial to improve the safety assessment of new pharmacological agents and optimize therapeutic strategies.

LACK OF STANDARDIZED ASSESSMENT PROTOCOLS

One major challenge in the study of drug-induced splenic alterations is the absence of universally accepted, standardized protocols for assessment. Histopathological evaluation methods, scoring systems for splenic damage, and criteria for defining splenic toxicity vary widely between laboratories and studies[16]. This inconsistency limits the comparability of findings across preclinical and clinical research, complicating risk assessment and regulatory decisions.

Furthermore, while routine histology remains the gold standard, integration of advanced techniques such as immunohistochemistry, flow cytometry, and molecular profiling is not standardized, reducing sensitivity and specificity for detecting subtle or early splenic changes. There is a need for validated, reproducible protocols incorporating quantitative morphometry and functional assays to better characterize drug-induced effects on splenic architecture and immune cell populations.

TRANSLATIONAL LIMITATIONS FROM ANIMAL MODELS TO HUMANS

[17]Animal models are indispensable for studying splenic toxicity and protective interventions; however, translational limitations pose a significant hurdle. Rodents, the most commonly used species, have spleen anatomy and immune cell distributions that differ in important ways from humans. For example, the proportion of white pulp to red pulp, presence of marginal zones, and splenic stromal composition vary, influencing responses to xenobiotics.

[18]Moreover, species-specific differences in drug metabolism, immune system complexity, and lifespan complicate extrapolation of findings to human clinical scenarios. Many drugs that induce splenic toxicity in animals do not produce equivalent effects in humans, and vice versa. This translational gap underscores the need for improved models, such as humanized mice, organ-on-chip systems, and in vitro spleen tissue cultures that better mimic human physiology.

Additional Knowledge Gaps

- **Mechanistic Insights:** Although oxidative stress, inflammation, and immune modulation are recognized as key mechanisms, detailed molecular pathways underlying drug-induced splenic damage remain incompletely understood. Identifying biomarkers predictive of splenic toxicity is an ongoing challenge.
- **Long-Term Effects:** Most studies focus on acute or subacute splenic changes, with limited data on chronic drug exposure effects and potential for recovery or fibrosis.
- **Clinical Monitoring:** Reliable non-invasive techniques for monitoring splenic health during drug therapy in patients are lacking, limiting early detection of adverse effects.

FUTURE DIRECTIONS AND EMERGING APPROACHES

The field of drug-induced splenic toxicity research is rapidly evolving with the integration of cutting-edge technologies and innovative experimental models. These advancements promise to overcome current challenges, enabling more accurate, mechanistic, and human-relevant assessments of splenic drug effects. Below are key future directions shaping the landscape of spleen research.

USE OF ADVANCED IMAGING AND OMICS TECHNOLOGIES

[19]Traditional histopathology, while invaluable, provides only static snapshots of splenic architecture. Advanced imaging modalities allow dynamic and non-invasive visualization of the spleen's microstructure and function in live subjects. Techniques like **high-resolution MRI** provide detailed anatomical images with contrast agents highlighting vascular and lymphoid components. **Multiphoton intravital microscopy** enables real-time imaging of immune cell interactions and trafficking within the spleen, providing insight into how drugs alter immune responses at the cellular level.

Furthermore, **molecular imaging** approaches such as PET combined with targeted radiotracers can quantify inflammation and metabolic activity in the spleen during drug exposure, offering biomarkers for toxicity severity and progression.

[20]Complementing imaging, **omics technologies**—including **genomics, transcriptomics, proteomics, and metabolomics**—enable a systems-level understanding of drug-induced changes. For instance, transcriptomic profiling of splenic tissue after chemotherapy can identify genes involved in apoptosis, immune suppression, or fibrosis. Proteomic studies reveal alterations in cytokine networks and signaling pathways, while metabolomics uncovers shifts in oxidative stress markers and energy metabolism.

International Journal of Pharmaceutical research and Applications
Volume 10, Issue 3, May-June 2025, pp:903-914 www.ijprajournal.com ISSN: 2456-4494

Integration of multi-omics data using bioinformatics and network analysis can identify novel therapeutic targets and predictive biomarkers for splenic toxicity, enabling personalized medicine approaches.

SPLEEN-ON-CHIP AND ORGANOID MODELS

[21]To bridge the translational gap between animal studies and human biology, **organ-on-chip** platforms and **3D organoids** provide promising alternatives. These microengineered devices recreate the spleen's complex microenvironment by integrating human immune cells (e.g., B cells, T cells, macrophages), endothelial barriers, and extracellular matrix components under controlled fluidic conditions [22]Spleen-on-chip models facilitate precise control over drug dosing, shear stress, and immune cell trafficking, allowing mechanistic studies of drug effects on immune function, cell viability, and inflammatory responses. For example, chips mimicking marginal zones have been used to study how biologics alter lymphocyte activation and migration.

[23]Similarly, **human spleen organoids** derived from induced pluripotent stem cells (iPSCs) or primary progenitors recapitulate key splenic structures such as white pulp follicles and red pulp vasculature. These organoids enable long-term culture and testing of chronic drug exposure effects, which are challenging in vivo.

Such in vitro models reduce animal use, improve human relevance, and expedite high-throughput screening of drug candidates and protective agents.

INTEGRATION INTO PRECLINICAL SAFETY EVALUATION

[24]Regulatory agencies increasingly recognize the value of these emerging technologies in augmenting traditional toxicology studies. Incorporating advanced imaging and spleen-specific omics endpoints in **preclinical safety pharmacology** can enhance early detection of immunotoxicity and splenic damage.

Moreover, combining spleen-on-chip and organoid data with in vivo studies can improve risk assessment accuracy, enabling better prediction of human responses. Such integrated approaches support **mechanistic toxicology**—understanding not just if a drug is toxic, but how and why it affects splenic function.

The development of **standardized protocols and validation frameworks** for these novel tools is underway, aiming to facilitate their acceptance in regulatory submissions and drug approval processes.

ARTIFICIAL INTELLIGENCE AND PREDICTIVE MODELING

[26]The future also lies in **artificial intelligence (AI)** and machine learning approaches that analyze large datasets generated from omics and imaging studies to predict drug-induced splenic toxicity. AI models can identify complex patterns and interactions that human analysis may miss, enabling early screening of compound libraries and personalized risk profiling.

PERSONALIZED MEDICINE AND THERAPEUTIC IMPLICATIONS

Advanced diagnostic tools emerging from these approaches will enable clinicians to monitor splenic health dynamically, adjust drug regimens accordingly, and employ spleen-protective adjunct therapies. Personalized interventions based on patient-specific genetic and immunological profiles will reduce adverse effects and improve treatment efficacy.

II. CONCLUSION

The spleen serves as a vital organ in immune surveillance, hematopoiesis, and blood filtration, making it highly susceptible to pharmacological interventions. This review highlights the diverse mechanisms through which drugs induce splenic alterations, including direct cytotoxicity, oxidative stress, immunomodulation, and vascular remodeling. These changes not only compromise splenic structure and function but also have broader implications for systemic immunity and patient safety.

Advancements in experimental models, imaging techniques, and omics technologies have significantly enhanced our ability to detect and characterize drug-induced splenic toxicity. Furthermore, innovative in vitro platforms such as spleen-on-chip and organoid models offer promising human-relevant alternatives for preclinical safety assessment. Integration of these emerging approaches with traditional methodologies is essential for improving translational accuracy and optimizing therapeutic strategies.

Continued spleen-focused pharmacological research is imperative to fill existing knowledge gaps and develop standardized evaluation protocols. Such efforts will facilitate the identification of spleen-protective agents and contribute to the

design of safer drugs. Ultimately, safeguarding splenic health during pharmacotherapy is crucial for maintaining immune competence and improving clinical outcomes.

[1]. Bronte, V., &Pittet, M. J. (2013). The spleen in local and systemic regulation of immunity. *Immunity*, *39*(5), 806–818. https://doi.org/10.1016/j.immuni.2013.10.010

[2]. Cesta, M. F. (2006). Normal structure, function, and histology of the spleen. *Toxicologic Pathology*, *34*(5), 455–465. https://doi.org/10.1080/01926230600867743

[3]. Mebius, R. E., & Kraal, G. (2005). Structure and function of the spleen. *Nature Reviews Immunology*, *5*(8), 606–616. https://doi.org/10.1038/nri1669

[4]. Wolfe, D., Kanji, S., Yazdi, F., et al. (2011). Drug-induced splenic injury: A review. *Canadian Journal of Gastroenterology*, *25*(7), 405–410.

[5]. Klein, U. (2000). The role of the spleen in the immune system and its significance in pharmacology. *Immunopharmacology*, *48*(2), 125–132.

[6]. Luster, M. I., Germolec, D. R., & Burleson, G. R. (1992). Immunotoxicology: Evaluation of immune system damage. *Toxicologic Pathology*, *20*(1), 126–136.

[7]. Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2001). Long-circulating and target-specific nanoparticles: Theory to practice. *Pharmacological Reviews*, *53*(2), 283–318.

[8]. El-Sayyad, H. I., Ismail, M. F., Shalaby, F. M., Abou-El-Magd, R. F., Gaur, R. L., Fernando, A., & Raj, M. H. (2009). Histopathological effects of doxorubicin and resveratrol on the liver and spleen of rats. *Toxicologic Pathology*, *37*(1), 100–114.

[9]. Abdel-Daim, M. M., Abuzead, S. M., Halawa, S., & El-Naga, R. N. (2019). Protective effects of quercetin and curcumin on doxorubicin-induced nephrotoxicity in rats. *Phytomedicine*, *49*, 1–8. https://doi.org/10.1016/j.phymed.2018.11.003

[10]. Al-Muzafar, H., & Amin, F. U. (2017). Chemotherapy-induced toxicity: Protective effects of natural products. *Journal of Cancer Research and Therapeutics*, *13*(5), 789–796. https://doi.org/10.4103/jcrt.JCRT_380_16

[11]. Chen, D. S., &Mellman, I. (2017). Elements of cancer immunity and the cancer-immune set point. *Nature*, *541*(7637), 321–330. https://doi.org/10.1038/nature21349

[12]. Kroemer, G., Galluzzi, L., & Brenner, C. (2015). Mitochondrial membrane permeabilization in cell death. *Physiological Reviews*, *87*(1), 99–163. https://doi.org/10.1152/physrev.00013.2006

[13]. Rauniyar, S., Khadka, D., & Shrestha, A. (2020). Spleen protective effect of *Ipomoea digitata* tuber extract against doxorubicin-induced toxicity in rats. *Journal of Ethnopharmacology*, *253*, 112638. https://doi.org/10.1016/j.jep.2020.112638

[14]. Sharma, V., Kumar, P., & Singh, D. (2018). Protective role of *Ipomoea digitata* tuber extract in chemotherapy-induced spleen toxicity. *Pharmacognosy Magazine*, *14*(56), 394–400. https://doi.org/10.4103/pm.pm_33_18

[15]. Davies, J. M., Lewis, M. P., Wimperis, J., Rafi, I., Ladhani, S., & Bolton-Maggs, P. H. B. (2011). Review of guidelines for the prevention and treatment of infection in patients with an absent or dysfunctional spleen: Prepared on behalf of the British Committee for Standards in Haematology. *British Journal of Haematology*, *155*(3), 308–317. https://doi.org/10.1111/j.1365-2141.2011.08740.x

[16]. Fayad, Z. A., Semelka, R. C., &Woosley, J. T. (2018). Imaging of splenic abnormalities. *Radiologic Clinics of North America*, *56*(5), 887–906. https://doi.org/10.1016/j.rcl.2018.06.009

[17]. Patel, M., Kumar, S., & Sharma, V. (2019). The spleen as a target organ in preclinical safety assessment: Importance and evaluation strategies. *Toxicologic Pathology*, *47*(6), 703–713. https://doi.org/10.1177/0192623319877134

[18]. Rheumatoid Arthritis Study Group. (2017). Autoimmune complications linked with immunomodulatory therapies: Role of the spleen. *Autoimmunity Reviews*, *16*(10), 1075–1083. https://doi.org/10.1016/j.autrev.2017.08.002

[19]. Li, X., & Wang, Z. (2020). Challenges in translational toxicology: Bridging the gap between animal models and human immune responses. *Toxicology Letters*, *332*, 11–19. https://doi.org/10.1016/j.toxlet.2020.06.009

[20]. Singh, R., Sharma, P., &Verma, A. (2021). Advances and challenges in histopathological evaluation of drug-induced organ toxicity.

Journal of Pharmacological Methods, *187*, 106531. https://doi.org/10.1016/j.jphm.2021.106531

[21]. Kim, S. Y., Park, J. H., & Lee, J. H. (2023). Microfluidic spleen-on-chip models for immunotoxicity screening. *Lab on a Chip*, *23*(5), 980–993. https://doi.org/10.1039/D2LC01034B

[22]. Lee, J. H., Kim, S., & Park, K. (2021). Advances in spleen-on-a-chip and organoid models for immunotoxicity testing. *Biomedical Microdevices*, *23*(4), 85. https://doi.org/10.1007/s10544-021-00550-2

[23]. Martinez, R., Gomez, L., & Torres, A. (2022). Human spleen organoids as a model for studying immune response and drug toxicity. *Stem Cell Reports*, *17*(6), 1321–1335. https://doi.org/10.1016/j.stemcr.2022.04.009

[24]. Tian, Y., Zhang, Q., & Wang, L. (2021). Intravital multiphoton microscopy reveals immune cell dynamics in the spleen. *Nature Communications*, *12*(1), 1234. https://doi.org/10.1038/s41467-021-21487-x

[25]. Wang, H., Zhang, L., & Zhou, Y. (2022). Multi-omics approaches in toxicology: Toward a comprehensive understanding of organ-specific drug effects. *Frontiers in Pharmacology*, *13*, 847356. https://doi.org/10.3389/fphar.2022.847356

[26]. Zhou, X., Li, W., & Chen, M. (2023). Artificial intelligence for predictive toxicology: Applications in drug-induced spleen injury. *Computational Toxicology*, *28*, 100295. https://doi.org/10.1016/j.comtox.2023.100295