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Evaluating Systemic Immuno-Inflammatory Markers as Indicators of Early Hepatotoxicity Induced by *Andrographis paniculata* Ethanol Leaf Extract in Rats

Anyalebechi, Eberechukwu Okwuchi^{1*}, Nwogu, Arit Okechukwu¹ and Elekima, Ibioku^{1,2}
Department of Clinical Chemistry, Rivers State University, Port Harcourt, Nigeria¹, Department of Medical Diagnostics, Cranfield University, Bedford, United Kingdom².

Author for Correspondence*: anyalebechi.eberechukwu@ust.edu.ng/+234-806-075-8003

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Abstract

Andrographis paniculata is a widely used medicinal plant in traditional medicine, for its anti-inflammatory and several therapeutic properties. However, its potential hepatotoxic effects remain insufficiently explored. This study evaluated the impact of graded doses of *A. paniculata* ethanolic leaf extract on inflammatory markers, immunological indices and liver histology in Wistar rats. Twenty-one male Wistar rats were divided into three groups (n = 7): a control group receiving no treatment, a low-dose group administered 10 mg/kg, and a high-dose group administered 200 mg/kg of ethanolic extract orally for 14 days. Full blood count parameters were used to compute neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation indices (SII, SIRI). Liver samples were examined histologically using H&E staining. A significant reduction in NLR (p=0.0403) and significant increase in PLR occurred in the low-dose group, suggesting an immune-modulatory effect, whereas MLR, SII, and SIRI showed no significant changes across groups. Red cell distribution width (RDW) significantly decreased in both treated groups compared to controls (p=0.0097). Despite minimal haematological disruption, dose-dependent hepatic alterations including hepatocyte vacuolation, sinusoidal dilation, and pyknotic nuclei, were observed in treated groups, more pronounced at higher dose. Although *A. paniculata* extract demonstrated mild immunomodulatory effects, its administration induced observable hepatic tissue damage even at low dose within short-term exposure.

Haematological inflammatory markers alone may not reliably predict early hepatic injury following herbal extract consumption. Concurrent histopathological assessment is essential when evaluating safety of medicinal plants.

Keywords: *Andrographis paniculata*, immunological markers, inflammatory markers, hepatotoxicity, Wistar rats, liver histology.

Introduction

Andrographis paniculata is a herbal plant that thrives in subtropical countries like Nigeria, India, China, Vietnam, Malaysia, Japan, Thailand, and in Scandinavia (Ayenitaju and Omokhuale, 2023; Parthasarathy and Prince, 2023). Because of its extreme bitter taste and use as bitter tonic, it is called “King of Bitters” (Bhaisare *et al.*, 2023). *Andrographis paniculata* has been long used as a therapeutic plant in Traditional Chinese, Ayurveda, and other herbal medicinal practices due to its enormous pharmacological effects resulting from the phytochemical components found in the plant, including Diterpenes, alkenes, ketones, aldehydes, Tannins, Flavonoids, Cyanogenic Glycoside, Phenol, Reducing Sugar, Alkaloids and Saponins (Owoade *et al.*, 2021; Anyalebechi *et al.*, 2025). *Andrographis paniculata* whole plant, stem, leave, and roots have long been used in traditional medicine for the treatment of several conditions such as stomach pain, inflammation, intermittent fever, malaria, cancer, diabetes, hypertension, respiratory infections and influenza (Eziefula *et al.*, 2024).

Red blood cell distribution width (RDW) is a parameter measured automatically in every full blood count (FBC) that actually shows the degree

of anisocytosis of red blood cells. It is a cheaper technique used in everyday clinical practice together with other parameters for the diagnosis and determination of the cause of anaemia (Katsarosa *et al.*, 2020). In different pathological conditions, such as cardiovascular diseases, autoimmune diseases, malignancy, chronic renal diseases and chronic respiratory diseases, where inflammation and oxidative stress comprise the major pathophysiological insults, red cell distribution width acts as a significant and competent marker for prediction and assessment of disease activity and severity (Pathak *et al.*, 2017). Elevated RDW is associated with inflammation (Vayá *et al.*, 2015) and is linked to an increased risk of morbidity and mortality in conditions where inflammation is a major factor, such as cardiovascular diseases, chronic kidney disease, and sepsis (Fornal *et al.*, 2025).

Systemic immune inflammation (SII) index is a novel immune-inflammation-related index, and a comprehensive combination based on the peripheral lymphocyte, neutrophil, and platelet counts. SII is calculated as platelet count x neutrophil/lymphocyte count (Hu *et al.*, 2014; Zhang *et al.*, 2019). It has been suggested that since this formula includes 3 cell types, it may provide valuable information regarding the immune system and inflammation (Candemir *et al.*, 2021). The SII has been shown to be a useful marker primarily for predicting poor clinical outcomes in cancer patients and inflammatory diseases (Yang *et al.*, 2018).

Immunological markers serve as valuable tools for assessing immune responses to threats and evaluating the efficacy of treatment interventions. They provide a dynamic and comprehensive means of understanding disease mechanisms (Kaushik *et al.*, 2025).

Ratios of different types of white blood cells, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and monocyte-to-lymphocyte ratio (MLR) can reflect the balance of the immune response. They are investigated as predictors of prognosis in many diseases (Bozan *et al.*, 2016; Mercan *et al.*, 2016; Chen *et al.*, 2020; Chen *et al.*, 2020). The neutrophil-to-lymphocyte ratio (NLR) is obtained from the ratio of absolute neutrophil and absolute lymphocyte counts of a complete blood

count (Singh *et al.*, 2023). High NLR indicates the increased intensity of the inflammatory response and damage to the immune system, respectively (Zhuang *et al.*, 2020). The platelet-to-lymphocyte ratio (PLR) represents the ratio of thrombopoiesis and general inflammation, both of which participate in fibrosis of the liver (Kumar *et al.*, 2025). It is clinically significant in hepatic disorders and serves as an indicator of liver fibrosis, cirrhosis, and overall inflammation. A high PLR can predict increased risk of developing cirrhosis in conditions like Nonalcoholic Fatty Liver Disease (NAFLD) and is associated with poor prognosis and survival in hepatocellular carcinoma (Ma *et al.*, 2016). The monocyte-to-lymphocyte ratio (MLR) serves as a useful inflammatory marker for various conditions, including cardiovascular disease, stroke, kidney injury, and certain cancers. A high MLR is associated with increased inflammation and can be a prognostic indicator for adverse outcomes in such conditions (Kocak *et al.*, 2020; Qiu *et al.*, 2023).

Studies that have evaluated the relationship between immunological/inflammatory haematological indices and histopathological changes following administration of *A. paniculata* ethanolic leaf extract are limited. Since the liver plays a central role in biotransformation and detoxification of drugs (Sumadewi, 2023), it is the primary target of herbal-induced organ injury (Seeff *et al.*, 2015; Parthasarathy and Prince, 2023). This study therefore investigates the effects of graded doses of *A. paniculata* ethanolic leaf extract on inflammatory and immunological markers, as well as liver histology in Wistar rats.

Materials and Methods

Plant materials and extract preparation

The fresh leaves of *A. paniculata* were bought from Mile 1 market, Port Harcourt, Rivers State, Nigeria. Identification and authentication of the plant was carried out by Dr. M. G. Ajuru and a specimen was deposited in the herbarium with voucher number RSUPb0178 at the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria. The fresh leaves were washed in clean water, air-dried at room temperature to remove moisture, and grinded into powder. Three thousand grams of the powdered *A. paniculata* leaves was soaked in 6000 ml of ethanol for 72 hours, afterwards, the excess solvent was evaporated using a rotary evaporator to a constant weight and dryness.

The dry crude extract was dissolved in appropriate amount of distilled water, prior to administration of the required doses.

Laboratory Animals

Male Wistar rats (21) with mean weight of 200 g were obtained from the Department of Plant and Animal Biology, Rivers State University, Port Harcourt, Nigeria. The rats were acclimatized in a room temperature maintained at 26-29 °C with a 12-h light-dark cycle for 2 weeks with free access to food and water ad libitum.

Experimental design

Twenty-one rats were divided into three groups of seven animals in compartmentalized cages.

- Group 1 (control rats) received no treatment except distilled water and rat feed.
- Group 2 rats received low dose *A. paniculata* ethanol extract at 10mg/kg body weight per oral for 14 days.
- Group 3 rats received high dose *A. paniculata* ethanol extract at 200mg/kg body weight per oral for 14 days.

At the end of the treatment period, all Wistar rats,

including the control group rats were anaesthetized using chloroform after overnight fast. Blood samples were obtained through cardiac puncture for laboratory analysis.

Determination of Inflammatory and Immunological markers

Blood samples collected into Ethylene Diamine Tetra-acetic acid (EDTA) bottles were analysed for Full Blood Count (FBC) using Mindray BC-5000 Autoanalyzer. The blood count parameters obtained were used for the calculation of NLR, LMR, PLR, SII and SIRI values respectively.

Histological study

The liver organs from the sacrificed Wistar rats were immediately fixed in 10% formal saline for histological studies using the H&E staining technique.

Statistics

The data obtained were analyzed using SPSS Software Version 24, for descriptive analysis, One-way analysis of variance (ANOVA) and t-test, at 95% confidence interval and p<0.05 was considered statistically significant.

Result

Table 1: Level of Immunological Markers

	NLR	MLR	PLR
Group 1 (Control) n = 5	0.49 ± 0.10 ^b	0.06 ± 0.02	46.49 ± 15.23 ^b
Group 2 (Low Dose) n = 5	0.29 ± 0.09 ^a	0.10 ± 0.07	93.73 ± 19.19 ^a
Group 3 (High Dose) n = 5	0.35 ± 0.08	0.05 ± 0.02	91.26 ± 67.89
F-value	4.928	1.452	1.975
p-value	0.0403 [*]	0.2898	0.2009

Keys:

NLR = Neutrophil-to-Lymphocyte ratio

MLR = Monocyte-to-Lymphocyte ratio

PLR = Platelet-to-Lymphocyte ratio

*=statistically significant

n= number of samples

a = statistically different from control group

b = statistically different from low dose group

Table 2: Level of Inflammatory markers

	RDW (%)	SII (Cells/L)	SIRI
Group 1 (Control) n = 5	43.00 ± 6.50 ^{bc}	120.2 ± 79.62	117.2 ± 38.69
Group 2 (Low Dose) n = 5	33.28 ± 2.29 ^a	141.6 ± 61.38	126.9 ± 67.36
Group 3 (High Dose) n = 5	32.08 ± 2.38 ^a	156.0 ± 24.07	86.55 ± 30.89
F-value	8.104	0.2915	0.5863
p-value	0.0097*	0.7547	0.5786

Keys:

RDW = Red blood cell distribution width

SII = Systemic immune inflammation index

SIRI = Systemic immune-inflammatory response index

*=statistically significant

n= number of samples

^a = statistically different from control group

^{bc} = statistically different from low dose group and high dose group

Table 3: Level of Blood Cell Indices

	TWBC (10 ⁹ /L)	NC (10 ⁹ /L)	LC (10 ⁹ /L)	MC (10 ⁹ /L)	PC (10 ⁹ /L)
Group 1 (Control) n = 5	8.343 ± 4.011	30.33 ± 4.071 ^b	63.08 ± 4.355 ^b	3.825 ± 1.037	268.8 ± 196.1
Group 2 (Low Dose) n = 5	7.513 ± 2.203	20.33 ± 5.778 ^a	70.88 ± 3.320 ^a	7.175 ± 4.969	490.0 ± 146.9
Group 3 (High Dose) n = 5	8.703 ± 4.753	24.63 ± 3.917	70.57 ± 5.064	3.467 ± 1.007	373.8 ± 166.1
F-value	0.1027	4.454	4.243	1.587	1.677
p-value	0.9035	0.0501	0.0554	0.2628	0.2403

Keys:

TWBC = Total White Blood Cell

NC = Neutrophil Count

LC = Lymphocyte Count

MC = Monocyte Count

C = Platelet Count

^a = statistically different from control group

^b = statistically different from low dose group

Histological Result

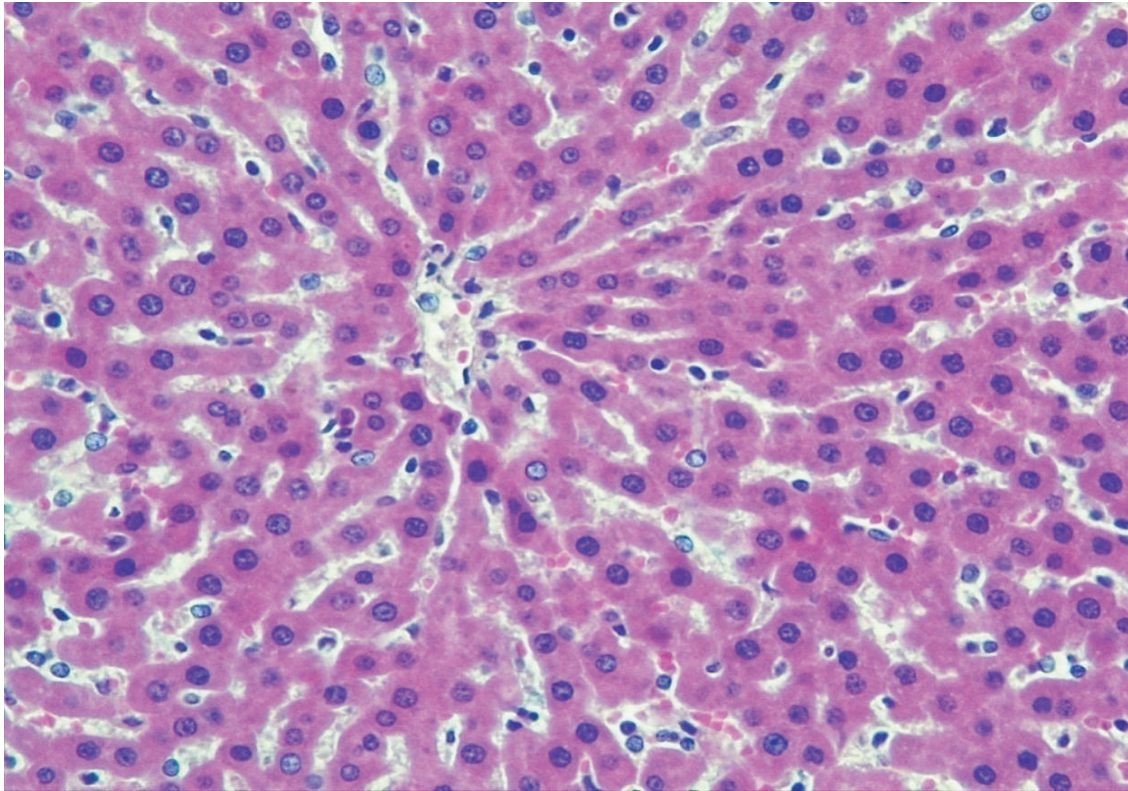


Figure 1: Photomicrograph section of liver tissue from control group. Section showed liver hepatocytes (black arrows), central vein (yellow arrows) and sinusoidal space (red arrows). (H&E X400).

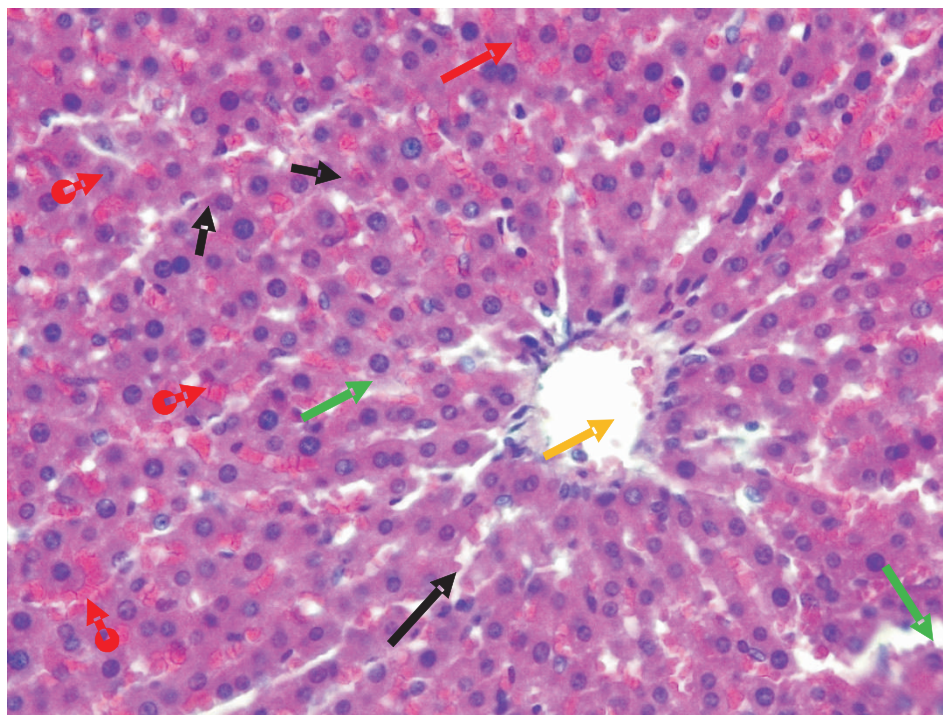


Figure 2: Photomicrograph section of liver tissue from group 2 (Low dose). Section showed mild liver injury with hepatocytes (red arrows), hepatocytes vacuolations (orange arrows), sinusoidal space expansions (green arrows) and general tissue extravasations (black arrows) (H&E, x400).

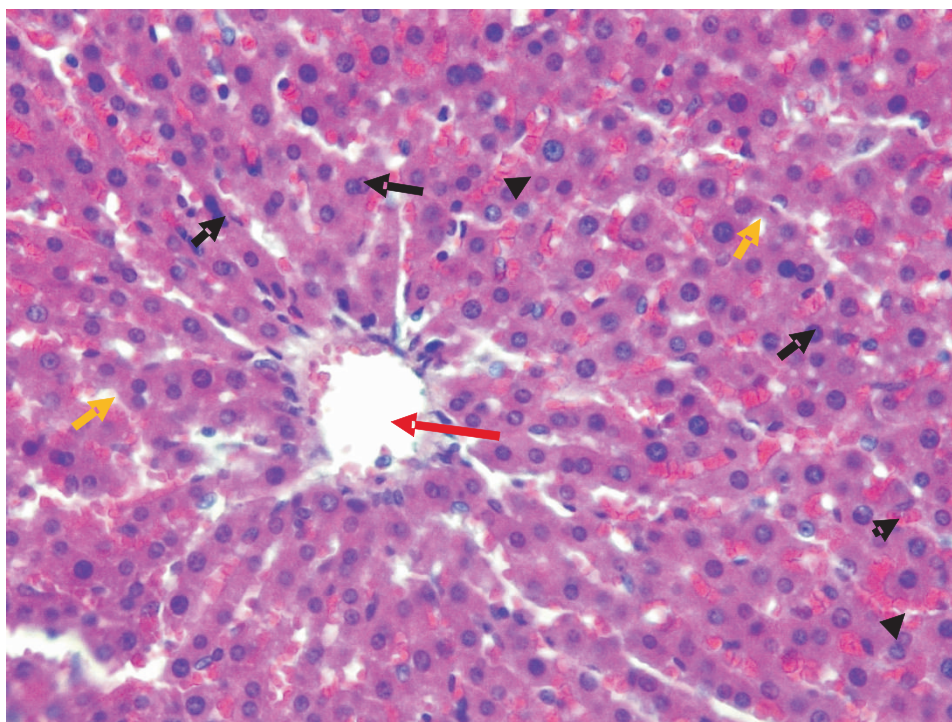


Figure 3: Photomicrograph section of liver tissue taken from group 3 (High dose). Section showed central vein (red arrow), some pyknotic hepatocytes nucleus (black arrows), cellular vacuolations (yellow arrows) and extravasation at the sinusoidal spaces (arrowhead). (H&E X400).

Discussion

The impact of *A. paniculata* ethanol extract on inflammatory markers, immunological indices and the liver histology in male Wistar rats was investigated in this study. The study revealed a dose-dependent effect of *A. paniculata* on inflammatory markers and histological alterations of the liver tissues, thereby indicating possible hepatotoxicity effects of *A. paniculata* regardless of its widely accepted therapeutic benefits.

Impact on Immunological Indices

The neutrophil-to-lymphocyte ratio (NLR) was significantly decreased in the low dose group compared with the control group. NLR is known as an effective marker for immune modulation and systemic inflammation (Singh *et al.*, 2023; Zhuang *et al.*, 2020; Badran *et al.*, 2025; Carollo *et al.*, 2025). NLR decrease in the low dose group may be suggestive of moderate response to immune-modulation or anti-inflammation at low *A. paniculata* ethanol extract concentrations. This observation is consistent with the literature where *A. paniculata* is reported to exhibit anti-inflammatory properties stemming from its phytochemical constituent andrographalide,

capable of attenuating recruitment of white blood cells and production of cytokines (Intharuksa *et al.*, 2022; Eziefule *et al.*, 2024; Shan *et al.*, 2025). On the other hand, monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR) showed no statistically significant difference across all groups. This implies that there were no substantial alterations in monocyte-mediated and platelet-mediated pathways of inflammation. This observation is consistent with the reports that PLR and MLR tend to primarily respond during chronic inflammatory diseases instead of acute inflammatory diseases (Ma *et al.*, 2016; Qiu *et al.*, 2023; Kumar *et al.*, 2025). There was significant increase in the low dose group, though not significant in the high dose group, in the level of PLR. This may signify compensatory thrombocyte response to mild hepatic stress, because PLR is usually associated with hepatic fibrosis and hepatic inflammatory disorders (Ma *et al.*, 2016).

Impact on Inflammatory Markers

This study observed a statistically significant decrease in red cell distribution width (RDW) in

the low dose and high dose treated groups compared to the control group respectively. Increased RDW typically occurs during oxidative stress and systemic inflammation (Vayá *et al.*, 2015; Katsarosa *et al.*, 2020). This decrease may be reflective of Inhibitory effect exerted by *A. paniculata* ethanol extract on inflammatory responses or inhibition of oxidative erythrocyte injury. This finding is consistent with the antioxidant activities of *A. paniculata* recorded in literature due to its phytochemical components such as diterpenoids and flavonoids capable of protecting the red blood cell membrane from oxidative damage (Owoade *et al.*, 2021).

The systemic immune inflammation index (SII) and the systemic inflammatory response index (SIRI) showed no significant differences across all groups. This implies that *A. paniculata* ethanol leaf extract did not cause widespread systemic inflammation during the 14 days treatment duration. SII and SIRI are both composite markers calculated from absolute neutrophil count, lymphocyte count, platelet count and monocyte count, usually utilized as prognostic indicators in conditions such as cancer, cardiovascular and chronic inflammatory diseases (Zhang *et al.*, 2019; Candemir *et al.*, 2021).

Impact on Hepatic Histology

Hepatic histological examination revealed hepatic alterations in the low dose and high dose groups, such as hepatocytes vacuolations, sinusoidal space expansions, pyknotic nuclei and general tissues extravasations. These features characterize response to hepatic stress and early hepatotoxicity (Sumadewi, 2023). The observed hepatic alterations align with the existing reports that herbal preparations including the ones considered to be traditionally safe, can cause hepatocellular injury via oxidative stress-related mechanisms, accumulation of bioactive compounds, or hepatic drug metabolizing enzymes interference (Seeff *et al.*, 2015). Despite the use of *A. paniculata* for its anti-inflammatory and hepato-protective potentials, at higher doses and long-term consumption it induces hepatocellular damage in experimental animals (Parthasarathy and Prince, 2023).

The hepatocytes vacuolations and pyknotic nuclei observed in the high dose group indicate early irreversible hepatic cell injury, which may be as a result of oxidative or metabolic overload caused by the extract. This observation is consistent with the findings from Anyalebechi *et al.* (2025) who reported changes in hepatic indices resulting from *A. paniculata* extract treatment.

Conclusion

This study observed dissociation between reduced RDW and increased hepatic abnormalities. The reduced RDW even in the presence of liver tissue injury indicates that RDW is not a reliable marker for early hepatic insult caused by short-term herbal exposures. This study has shown that *A. paniculata* ethanolic leaf extract exerts modulatory effects on certain inflammatory and immunological markers, as well as induces a dose-dependent hepatic histological alterations.

Recommendation

Immunological and inflammatory markers should be complemented with histological evaluation when assessing hepatotoxic effects, especially for herbal preparations comprising bioactive constituents that may exert differential systemic and organ-specific effects. There is need for dose regulation, long-term safety studies, and more advanced mechanistic exploration to understand the balance between *A. paniculata* therapeutic potential and hepatotoxic risk.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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