

APST**Asia-Pacific Journal of Science and Technology**<https://www.tci-thaijo.org/index.php/APST/index>Published by Research and Innovation Department,
Khon Kaen University, Thailand**Protective Effects of Antioxidant Extracts on Liver Damage and Electrical Properties in Mice Injected with Benzo[a]pyrene**Unggul P. Juswono^{1*}, Chomsin S. Widodo¹, Johan A. E. Noor¹, Risalatul Latifah¹, Nurdiana L. Sari¹ and Muhammad Faisal¹¹ Department of Physics, Brawijaya University, Malang, Indonesia* Corresponding author: unggul-pj@ub.ac.id

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Abstract

Benzo[a]pyrene (BaP) is a highly toxic polycyclic aromatic hydrocarbon that induces oxidative stress and structural damage in hepatic tissue. Antioxidant-rich plant extracts have been proposed as potential protective agents against BaP-induced liver injury. This study aimed to evaluate the protective effects of soursop leaf, garlic, and moringa leaf extracts against BaP-induced liver damage using complementary histopathological and electrical property analyses. Eighty-five male mice were randomly assigned to 17 treatment groups consisting of one untreated control group, one benzo[a]pyrene-only (BaP) group, and fifteen antioxidant treatment groups receiving BaP exposure followed by soursop leaf extract, garlic extract, or moringa leaf extract at five different dose levels. Liver tissue damage was assessed through microscopic histopathological examination, while electrical properties as impedance, resistivity, and dielectric constant were measured to evaluate cellular membrane integrity and tissue bioelectrical function. The results demonstrated that BaP exposure induced marked liver tissue damage and disrupted hepatic electrical properties. Antioxidant treatment groups showed significant structural and bioelectrical recovery compared to the BaP-only group. Among the tested treatments, antioxidant administration at intermediate doses produced the most consistent protective effects. Overall, antioxidant supplementation attenuated oxidative damage, improved hepatic tissue integrity, and restored electrical homeostasis in BaP-exposed mice. These findings indicate that soursop leaf, garlic, and moringa leaf extracts possess hepatoprotective potential against benzo[a]pyrene-induced toxicity and support the use of combined histopathological and electrical property analyses as sensitive indicators for evaluating liver tissue recovery.

Keywords: Antioxidants, liver, benzo[a]pyrene, electrical properties, microscopic images.**1. Introduction**

Benzo[a]pyrene (BaP) is a polycyclic aromatic hydrocarbon that is widely recognized as a potent environmental toxicant; it is generated by incomplete combustion processes, including vehicle emissions, industrial activities, and biomass burning [1]. Once absorbed into the body, BaP undergoes metabolic activation in the liver and generates reactive intermediates that induce oxidative stress, lipid peroxidation, and structural damage to hepatocytes [2]. Chronic exposure to BaP has been strongly associated with hepatic inflammation, cellular degeneration, and functional impairment, which makes the liver a primary target organ in BaP-induced toxicity [3,4].

In this study, the primary scientific focus is the biological evaluation of antioxidant interventions to mitigate benzo[a]pyrene-induced liver damage, given the growing need for safe, accessible, and effective protective strategies against environmental hepatotoxins [5]. Plant-derived antioxidants have attracted even greater attention due to their ability to neutralize reactive oxygen species (ROS), stabilize cellular membranes, and suppress oxidative damage at the tissue level. Natural antioxidant sources are particularly relevant in long-term exposure scenarios, where synthetic drugs may introduce additional toxic burdens [6].

Soursop leaves (*Annona muricata*), garlic (*Allium sativum*), and moringa leaves (*Moringa oleifera*) are well-documented medicinal plants that are rich in polyphenols, flavonoids, and organosulfur compounds with strong

antioxidant and anti-inflammatory activities [7]. Previous studies have demonstrated their hepatoprotective potential through biochemical and histological indicators, which include reduced lipid peroxidation, improved antioxidant enzyme activity, and attenuation of inflammatory responses [8]. The doses used in this study were determined based on established toxicological and pharmacological conversion principles; they adapted effective doses reported in previous *in vivo* studies to mice body weight to ensure physiological relevance while avoiding pro-oxidant effects at excessive concentrations. This dose-selection strategy has the aim of balancing efficacy and safety, a critical goal in antioxidant-based interventions [9–11].

Beyond conventional histopathological evaluation, tissue damage at the cellular level can also be reflected in changes in bioelectrical behavior [12]. In the present work, electrical properties as impedance, resistivity, and dielectric constant are employed as quantitative outcome measures to support and strengthen the biological assessment of antioxidant protection, rather than serving as the primary novelty of the study. Cellular membrane disruption caused by oxidative stress alters ion distribution and electrical polarization, which leads to measurable changes in tissue electrical properties. Recent studies have reported that antioxidant-mediated membrane stabilization can restore electrical homeostasis in damaged tissues; they suggest that bioelectrical parameters may serve as sensitive indicators of cellular recovery [11,13].

Recent experimental and systematic studies have increasingly demonstrated that antioxidant-mediated hepatoprotection is closely associated with preservation of cellular membrane integrity, which directly governs tissue bioelectrical behavior [14]. Antioxidant interventions using *Moringa oleifera* have been shown to attenuate hepatic oxidative stress and structural damage by suppressing lipid peroxidation and enhancing endogenous antioxidant enzymes, both in animal models and in cultured liver cells [15]. These protective effects are mechanistically linked to stabilization of hepatocyte membranes and maintenance of intracellular redox balance. Similarly, garlic (*Allium sativum*) supplementation has been extensively reported to improve liver function by modulating oxidative stress pathways, preserving membrane lipid composition, and maintaining hepatocellular integrity across various liver injury models. In parallel, *Annona muricata* leaf extract has demonstrated strong hepatoprotective capacity against chemically-induced toxicity by enhancing antioxidant enzyme activity and reducing lipid peroxidation, with additional evidence supporting its safety and tolerability *in vivo* [16]. Recent literature increasingly supports a direct link between antioxidant activity, membrane integrity, and tissue bioelectrical properties, particularly in organs exposed to oxidative stress, such as the liver. Improvements in impedance and dielectric behavior have been correlated with reduced lipid peroxidation, enhanced membrane protein organization, and recovery of intracellular ion balance following antioxidant treatment [17].

Despite extensive research on benzo[a]pyrene toxicity and the antioxidant potential of medicinal plants, there remains a clear research gap in studies that simultaneously evaluate liver protection using both histopathological assessment and bioelectrical property analysis within a single experimental framework. Specifically, comparative evaluation of multiple antioxidant plant extracts at controlled dose levels, combined with quantitative electrical characterization of liver tissue following BaP exposure, has not been sufficiently explored. This study aimed to address this gap by systematically investigating the protective effects of soursop leaf, garlic, and moringa leaf extracts against benzo[a]pyrene-induced liver damage, using microscopic histopathology as the primary biological endpoint and electrical property measurements as complementary quantitative indicators of tissue recovery. This integrated approach is expected to provide a more comprehensive understanding of antioxidant-mediated hepatoprotection and to strengthen the use of bioelectrical parameters as supportive tools in toxicological evaluation.

2. Materials and Methods

2.1 Experimental Animals, Ethical Approval, and Study Design

All experimental procedures involving animals were reviewed and approved by the Local Ethics Committee of Brawijaya University, Indonesia (Ethical approval number: 128-KEP-UB-2023, approved on August 21, 2023), and were conducted in full compliance with the Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines. Eighty-five healthy male mice (*Mus musculus*), aged 2-3 months and weighing 25-30 g, were used in this study. Animals were housed under standard laboratory conditions (temperature 22-25 °C, 12 h light/dark cycle) with *ad libitum* access to food and water. Prior to treatment, the mice were acclimatized for seven days to minimize stress-related bias.

The animals were randomly assigned to 17 treatment groups, each consisting of 5 mice. The experimental design comprised one untreated control group, one benzo[a]pyrene-only (BaP) group, and fifteen antioxidant treatment groups that received BaP exposure followed by antioxidant administration. The antioxidant groups received soursop leaf extract (*Annona muricata*), garlic extract (*Allium sativum*), or moringa leaf extract (*Moringa oleifera*) at five dose levels for each plant extract. To improve clarity and reduce redundancy, the detailed grouping scheme is summarized in Table 1, while dose ranges and treatment rationale are described textually in this section.

Table 1 Grouping of experimental animals.

Treatment description	Group code	Benzo[a]pyrene Dose (mg/kg BW)	Antioxidant Dose (mg/kg BW)
Negative control	Control	-	-
Positive control (Benzo[a]pyrene)	BaP	0.3	-
Benzo[a]pyrene + <i>Annona muricata</i> leaf antioxidant	KSR1; KSR2; KSR3; KSR4; KSR5	0.3	1.9; 2.9; 3.9; 4.9; 5.9
Benzo[a]pyrene + <i>Allium sativum</i> antioxidant	KBP1; KBP2; KBP3; KBP4; KBP5	0.3	1.1; 1.7; 2.3; 2.9; 3.5
Benzo[a]pyrene + <i>Moringa oleifera</i> leaf antioxidant	KKL1; KKL2; KKL3; KKL4; KKL5	0.3	2.9; 4.4; 5.9; 7.4; 8.9

Benzo[a]pyrene (BaP) was administered intraperitoneally for 14 days to induce liver damage. Antioxidant treatments were administered orally for 14 days following BaP exposure. Dose levels for each plant extract were selected based on previously published *in vivo* studies and principles of body-weight (BW) conversion [9–11,18].

2.2 Liver Tissue Collection and Preparation for Electrical Measurements

The mice were euthanized by approved cervical dislocation following light anesthesia, in accordance with ethical guidelines. Liver tissues were immediately excised, rinsed gently with cold physiological saline to remove residual blood, and analyzed in a fresh state without chemical fixation. Samples were taken from the same liver lobe region to minimize anatomical variability. Each liver sample was trimmed into rectangular sections with uniform dimensions (approximately 10 mm × 10 mm × 3 mm). Electrical measurements were performed at room temperature (25 ± 1 °C) within 15 minutes of tissue excision to prevent postmortem degradation effects.

Measurement of resistivity and dielectric constant on the liver samples used a Bioelectrical Impedance Analyzer (BIA) unit integrated with a Personal Computer (PC). Measurements were made by injecting a current of 10 μ A in a frequency range of 100 Hz to 100,000 Hz. A sample is placed between parallel plate electrodes and then connected to a Bioelectrical Impedance Analyzer (BIA) to measure impedance (Z), capacitance (C), and resistance (R). Prior to measurements, the BIA system was calibrated using standard resistive-capacitive reference materials to ensure measurement accuracy and reproducibility. Measurement results of capacitance values (C) and resistance values (R) were then used to find the dielectric constant (by Equation 1) and the resistivity value of the material (by Equation 2):

$$\epsilon_r = \frac{C.d}{\epsilon_0.A} \quad (1)$$

$$\rho = \frac{R.A}{d} \quad (2)$$

Cross-sectional area (A) and distance between parallel plates (d) were obtained from the results of previous measurements, while permittivity of air ($\epsilon_0 = 8.85 \times 10^{-12}$ F / m) was obtained from literature. These equations quantify intrinsic tissue electrical behavior and link electrical responses to cellular membrane integrity and ionic distribution [19].

2.3 Histopathological observation

Liver samples for histopathological analysis were collected from the same anatomical region as electrical measurements to ensure consistency across evaluation methods, specifically from the left lateral lobe. Tissues were fixed in 10% neutral buffered formalin for 24 h, dehydrated through graded ethanol solutions, cleared in xylene, embedded in paraffin, and sectioned at a thickness of 5 μ m using a rotary microtome. Sections were rid of paraffin in xylene, rehydrated through graded ethanol to water, stained using hematoxylin and eosin (H&E), and mounted with standard mounting media. Histological observations were performed using a binocular light microscope at 100× to confirm liver architecture (as the central vein and portal triad) and at 500× for cellular injury assessment and image acquisition. Histopathological features comprising hydropic degeneration (H), parenchymal degeneration (P), necrosis (Ne), and normal cells (N), were evaluated using the Manja Roenigk scoring method, and representative micrographs were captured with scale bars and morphological labels. The damage score for each field was calculated using Equation 3 below:

$$\text{Damage score} = \frac{(N \times 1) + (P \times 2) + (H \times 3) + (Ne \times 4)}{(N + P + H + Ne)} \quad (3)$$

The average damage value for a treatment group was obtained by summing the damage in each field of view and dividing by the number of samples in that treatment. The Manja Roenigk scoring method indicates that a

higher score indicates greater liver tissue damage, which allows for direct quantitative comparison between treatment groups [20].

2.4 Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate differences in antioxidant dose levels within each treatment series (KBP, KSR, and KKL) for impedance, resistivity, dielectric constant, and cell damage. Normality was tested prior to ANOVA using the Shapiro-Wilk test, and homogeneity of variance was checked using Levene's test. When the assumptions were met (Shapiro-Wilk $p > 0.05$ and Levene $p > 0.05$), parametric ANOVA results were reported. All statistical tests were two-tailed, and a p -value < 0.05 was considered statistically significant. The analysis was performed using Python (SciPy library) for all calculations [21].

3. Results

Biological tissues exhibit inherent bioelectrical properties that reflect cellular structure and metabolic activity. In this study, liver tissue damage induced by benzo[a]pyrene exposure was evaluated using measurements of impedance, resistivity, and dielectric constant as indicators of membrane integrity and cellular function. Figure 1 a-c presents the frequency-dependent impedance profiles of liver tissue from mice treated with antioxidants derived from *Allium sativum* (KBP), *Annona muricata* (KSR), and *Moringa oleifera* (KKL). Benzo[a]pyrene exposure resulted in a marked reduction in liver impedance compared to the control group, which indicates substantial membrane damage and increased ionic permeability. Administration of plant-derived antioxidants led to an overall increase in impedance values, which suggests partial restoration of membrane integrity. Across antioxidant sources, a consistent dose-response trend was observed: impedance increased from dose 1 to dose 3 and then plateaued or declined at higher doses. This indicates a shared pattern of maximal improvement at the intermediate dose.

For all three antioxidant sources, impedance values increased progressively up to the third dose, which consistently produced the highest impedance values and the closest approximation to the control group. However, full recovery was not achieved (Figure 1a-c). Statistical analysis demonstrated that impedance data for KBP, KKL, and KSR met normality assumptions based on the Shapiro-Wilk test of ANOVA residuals (KBP p -value = 0.986; KKL p -value = 0.057; KSR p -value = 0.432) and exhibited homogeneous variances (Levene test: KBP p -value = 0.837; KKL p -value = 0.969; KSR p -value = 0.995), which then permits parametric analysis. One-way ANOVA revealed that variations in antioxidant dose did not result in statistically significant differences in impedance values for KBP (p -value = 0.975), KKL (p -value = 0.999), or KSR (p -value = 0.947). Dose 3 consistently produced the highest impedance recovery; this pattern is consistent with improved membrane-related electrical behavior (as reduced ion leakage and better preservation of cellular barrier function) at intermediate dose levels.

In the KKL group, a notable dose-dependent reversal was recorded: the overdosing effect occurred at dose 5 (8.9 mg/kg BW), where impedance decreased markedly and fell below earlier dose levels (Figure 1c). This decline at dose 5 in the series (8.9 mg/kg BW) represents an important and distinctive finding compared with the more stable pattern observed at lower doses. It highlights that the maximal impedance recovery was not maintained at the highest dose. The overall trend across treatment groups, therefore, shows a consistent peak response at dose 3. In contrast, dose 5 for KKL showed the most pronounced reduction in impedance compared with prior doses within its series. Electrical properties of liver tissue were also assessed using resistivity ($\Omega \cdot m$), which reflects the intrinsic opposition of a material to electric current flow. Figure 1d-f shows dose-response profiles of liver resistivity for KSR (doses 1.9-5.9 mg/kg BW), KBP (doses 1.1-3.5 mg/kg BW), and KKL (doses 2.9-8.9 mg/kg BW), with five replicates at each dose. The control group exhibited the highest resistivity (24.79 Ωm), whereas the benzo[a]pyrene group showed the lowest resistivity (11.41 Ωm). Within antioxidant-treated groups, resistivity values ranged from 9.32 to 22.00 Ωm for KBP, 11.13 to 16.72 Ωm for KSR, and 9.81 to 21.61 Ωm for KKL, with the highest antioxidant-group resistivity being observed at dose 3 of *Moringa oleifera* (5.9 mg/kg BW). Normality testing on one-way ANOVA residuals yielded Shapiro-Wilk p -values of 0.060 (KSR), 0.0039 (KBP), and 0.066 (KKL), and one-way ANOVA indicated statistically significant differences across doses for each source: p -value = 6.35×10^{-9} (KSR), p -value = 4.88×10^{-6} (KBP), and p -value = 1.74×10^{-12} (KKL). In terms of trend interpretation, resistivity increased toward dose 3 and then decreased at higher doses in parts of the dataset, which is consistent with a peak response at the intermediate dose.

The dielectric constant was evaluated as an additional electrical property, and dose-dependent patterns for KBP, KSR, and KKL are shown in Figure 1g-i. Across antioxidant dosing, the first, second, and third doses produced lower dielectric constant values than the fourth and fifth doses, and the third dose produced the lowest dielectric constant value within each antioxidant series. Within antioxidant-treated groups, dielectric constant values ranged from 26,709.21 to 47,532.80 for KSR, 21,885.24 to 62,118.90 for KBP, and 15,278.05 to 61,297.60 for KKL (five replicates per dose). Normality testing on one-way ANOVA residuals yielded Shapiro-Wilk p -values of 0.073 (KSR), 0.0071 (KBP), and 0.316 (KKL). One-way ANOVA showed statistically significant

differences across doses for each source, with p-values of 2.91×10^{-8} (KSR), 0.00393 (KBP), and 2.67×10^{-11} (KKL). The trend that formed across all antioxidant types indicated that dose 3 produced the lowest dielectric constant values (closest to control), which was followed by increases at doses 4 and 5; this reinforces dose 3 as the most effective level across the measured electrical outcomes. Taken together across impedance (Figure 1a-c), resistivity (Figure 1d-f), and dielectric constant (Figure 1g-i), the integrated electrical profile consistently peaked at dose 3 (higher impedance and resistivity with lower dielectric constant relative to other doses). Conversely, the Moringa series showed a pronounced deviation at dose 5, where the electrical pattern shifted away from the dose-3 optimum.

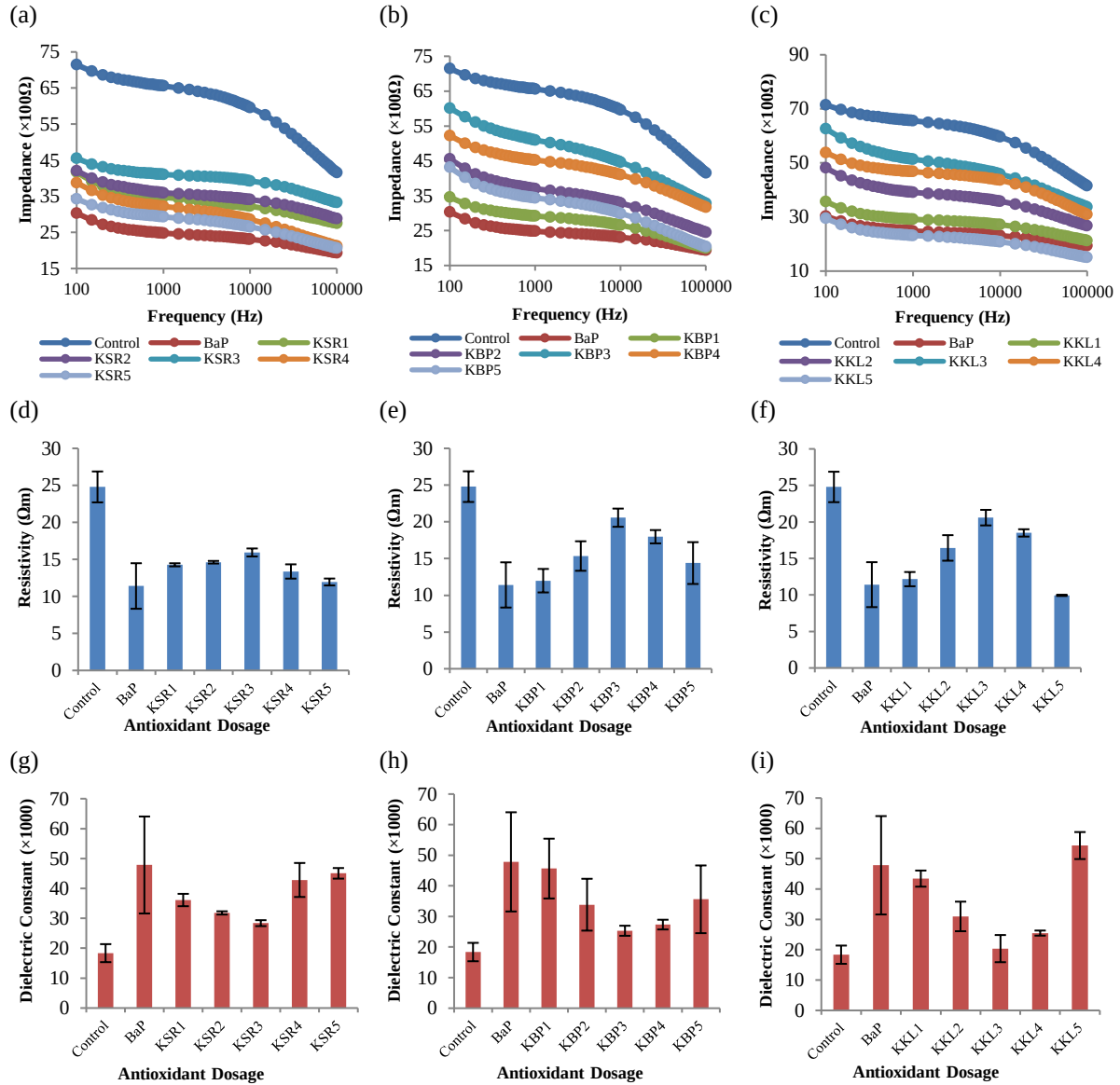


Figure 1 Characterization of electrical properties at various antioxidant doses: (a-c) impedance of KSR, KBP, KKL treatments; (d-f) resistivity of KSR, KBP, KKL treatments; (g-i) dielectric constant of KSR, KBP, KKL treatments.

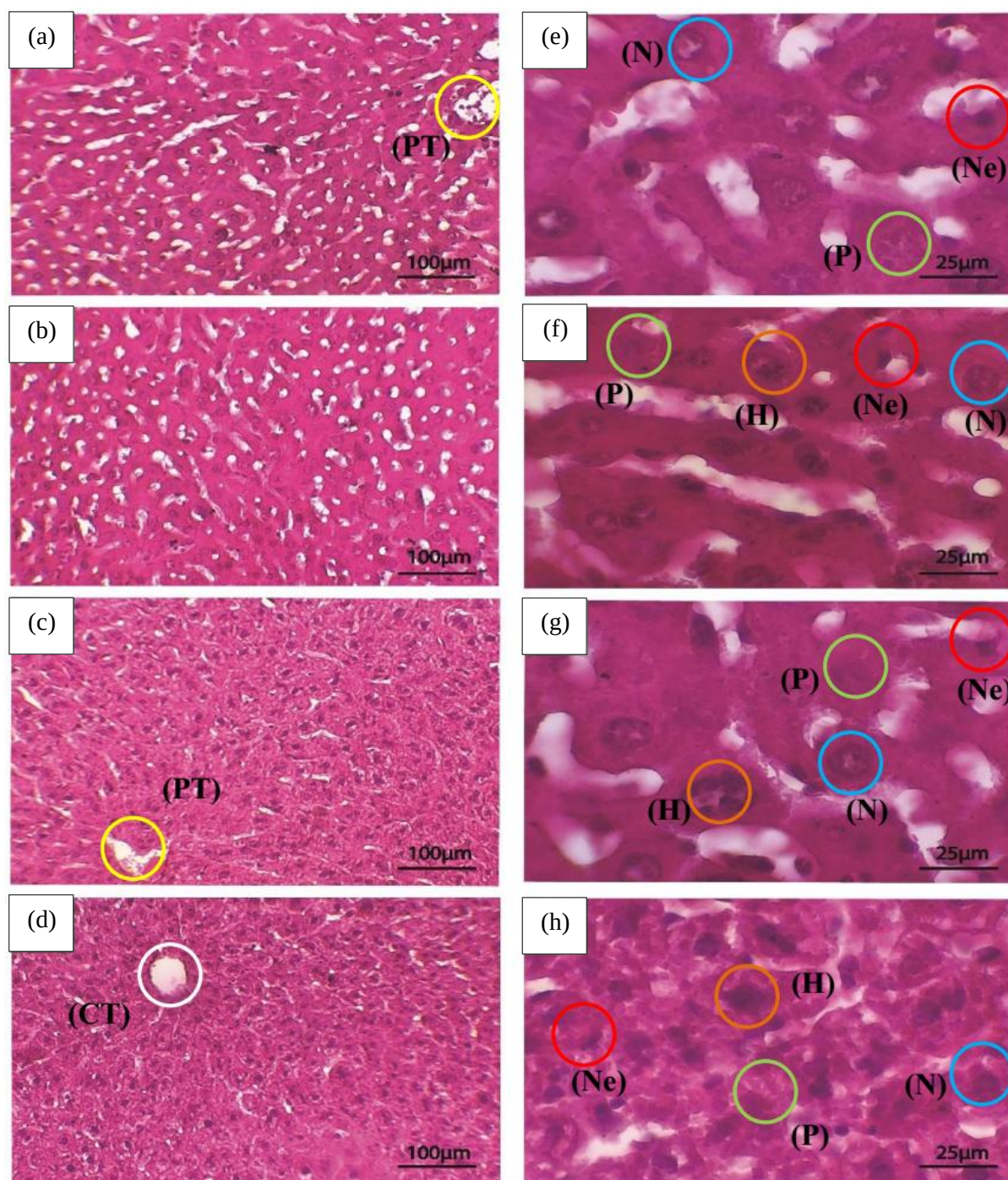


Figure 2 Liver histopathology: negative control, positive control, *Moringa oleifera* 5.9 mg/kg BW, and *Moringa oleifera* 8.9 mg/kg BW with 100× (a-d) and 500× (e-h) magnification. Labels: portal triad (PT), central vein (CT), hydropic degeneration (H), parenchymal degeneration (P), necrosis (Ne), and normal cells (N).

Histopathological observations of mice livers in Figure 2 support the data from measurements of cellular electrical properties, where administration of benzo[a]pyrene and antioxidants at varying doses can cause changes in the cells that make up the liver. Liver identity and architecture were confirmed by portal triads (PT) and central veins (CT) that are visible at low magnification (a-d) [22]. Negative controls showed largely normal hepatocytes with preserved lobular organization and uniform cell outline (a, e). Positive controls (benzo[a]pyrene) showed the highest levels of cell damage parameters (b, f), including hydropic changes (hepatocyte swelling and pale cytoplasm) and parenchymal degeneration (loss of characteristic cytoplasmic texture) [23,24]. The effect of antioxidant treatment on hepatocyte cells was the highest at a *Moringa oleifera* dose of 5.9 mg/kg BW and decreased again at a *Moringa oleifera* dose of 8.9 mg/kg BW. Good histological condition is characterized by many normal hepatocytes and few cells undergoing degeneration/necrosis. Histopathological observations

correlated with impedance, resistivity, and dielectric constant profiles. Impedance and resistivity values tended to increase, while dielectric constant values decreased when cells were in a healthy state. The *Moringa oleifera* 8.9 mg/kg BW group showed deviations from the treatment, which indicates an overdose effect. The dose-response pattern of *Moringa oleifera* 5.9 mg/kg BW antioxidant showed the most preserved cell morphology (c, g).

To determine the score of liver cell damage, the Manja Roenigk scoring method was used. Graphs of the accumulative liver damage scores of mice treated with antioxidants from *Annona muricata* (KSR), *Allium sativum* (KBP), and *Moringa oleifera* (KKL) are presented in Figure 3.

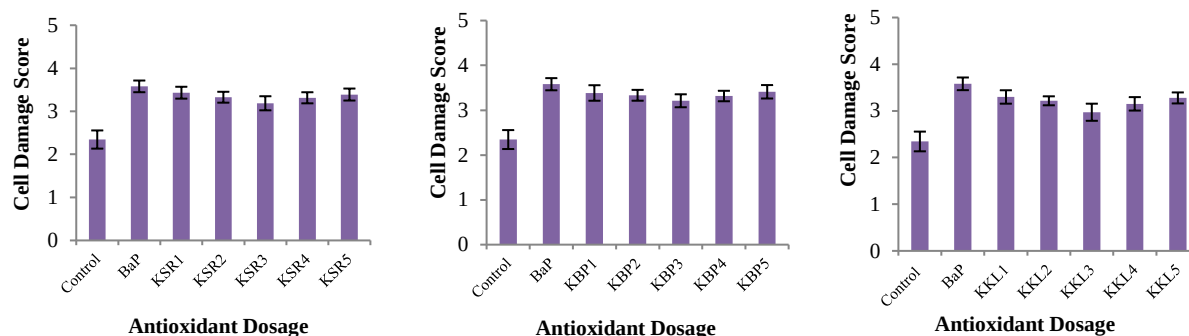


Figure 3 Graph of the relationship between antioxidant dose variations and liver cell damage scores.

Based on calculations using the Manja Roenigk scoring method, the control group showed the lowest damage scores, which indicates a higher proportion of healthy hepatocytes. In contrast, the benzo[a]pyrene group showed higher damage scores that are consistent with increased hepatocyte injury. Across antioxidant-treated groups, liver damage scores decreased relative to benzo[a]pyrene, which indicates reduced histopathological injury. Statistical analysis demonstrated that the damage score data met normality assumptions based on the Shapiro-Wilk test of ANOVA residuals (KSR p-value = 0.4189; KBP p-value = 0.0671; KKL p-value = 0.2200) and exhibited homogeneous variances (Levene test: KSR p-value = 0.2889; KBP p-value = 0.7123; KKL p-value = 0.6592) that support the use of parametric analysis. One-way ANOVA indicated that antioxidant dose variations produced statistically significant differences in liver damage scores for KSR (p-value = 4.44×10^{-4}), KBP (p-value = 0.0369), and KKL (p-value = 3.76×10^{-7}). Consistent with the dose-response pattern in Figure 3, the third dose showed the lowest damage scores across antioxidant sources, and the KKL series displayed the most pronounced reduction at the third dose. At the fourth dose, damage scores increased again relative to the third dose, while the overall histopathological findings continued to align with the observed dose-dependent profiles of the measured bioelectrical parameters.

Histopathological assessment and electrical measurements revealed a consistent dose-dependent pattern across antioxidant sources. In the microscopic assessment (Manja Roenigk), liver damage decreased after antioxidant administration, reaching the lowest value at 5.9 mg/kg BW, particularly for *Moringa oleifera* (KKL). In contrast, higher doses tended to reduce this improvement. In parallel, the electrical profile at the third dose showed the most favorable shift that is characterized by higher impedance and resistivity as well as a lower dielectric constant than the other doses. This agreement indicates that the observed changes at the cellular level are accompanied by measurable changes in the bioelectrical behavior of the tissue, which provides an integrated basis for interpreting the treatment effects.

4. Discussion

The liver cell membrane is composed of a lipid bilayer consisting of hydrophilic heads and hydrophobic lipid tails with embedded functional proteins. These membrane proteins act as selective channels and carriers, and they regulate ion transport and maintain intracellular homeostasis [25]. From a biological standpoint, the liver cell membrane functions as an active regulatory interface rather than a passive structural boundary. Exposure to benzo[a]pyrene induces oxidative stress that preferentially targets membrane lipids and proteins, which results in lipid peroxidation, altered membrane fluidity, and loss of selective permeability [26]. These molecular disruptions compromise the ability of hepatocytes to maintain electrochemical gradients, and they ultimately lead to functional impairment of the liver. The structural organization of the hepatocyte membrane, including polar membrane heads and non-polar lipid tails, is schematically illustrated in Figure 4.

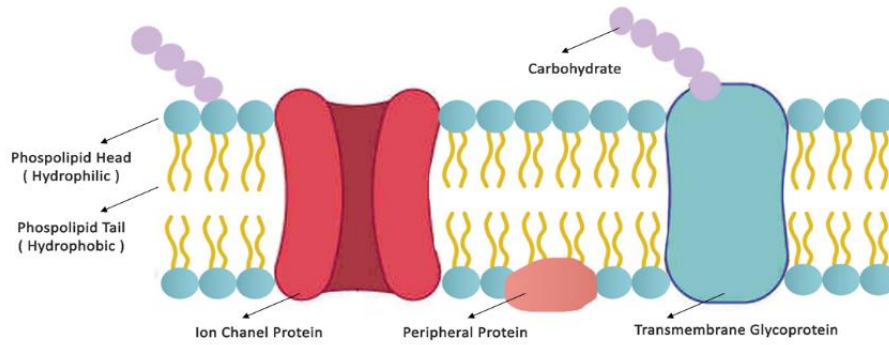


Figure 4 Cell membrane structure.

Such membrane-level damage has direct physiological consequences, including impaired ion transport, dysregulation of intracellular calcium homeostasis, and mitochondrial dysfunction. As membrane integrity deteriorates, hepatocytes become unable to maintain the electrochemical gradients that are essential for cellular viability. Antioxidant compounds derived from *Annona muricata*, *Allium sativum*, and *Moringa oleifera* mitigate these effects by scavenging reactive oxygen species, stabilizing membrane phospholipids, and preserving protein conformation. By limiting the oxidative disruption of the lipid bilayer, antioxidants help maintain membrane selectivity and ionic balance, which is expected to influence both tissue morphology and functional electrical behavior.

This biological context provides a logical foundation for interpreting liver tissue behavior using a bioelectrical framework. Based on the membrane architecture, the liver cell membrane can be conceptually modeled as a resistor-capacitor (RC) system, where the polar membrane heads act as capacitive elements that store charge, while the hydrophobic lipid core serves as a resistive barrier to ion flow [27]. This conceptual RC analogy, as illustrated in Figure 5, reflects a biological reality: intact membranes resist ionic penetration, whereas damaged membranes permit excessive ion leakage.

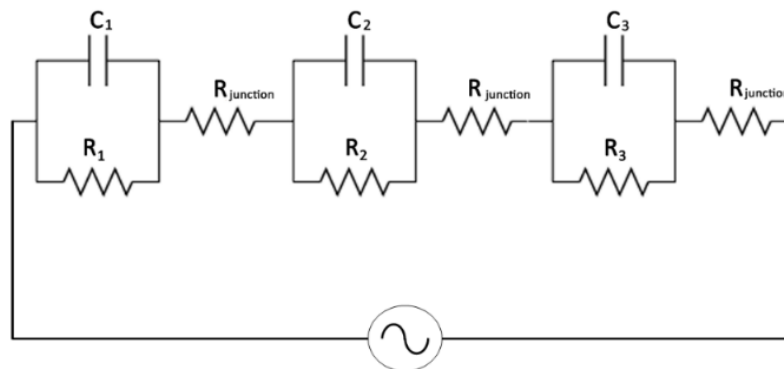


Figure 5 Equivalent circuit of a capacitor resistor in a cell membrane.

At low frequencies, electrical currents predominantly travel through extracellular spaces, while at higher frequencies, they penetrate cell membranes due to their capacitive properties. Thus, changes in impedance, resistivity, and dielectric constant can be interpreted as functional indicators of membrane integrity and cellular viability rather than abstract electrical parameters. In benzo[a]pyrene-exposed liver tissue, oxidative damage to peripheral and integral membrane proteins, as well as non-polar lipid domains, reduces resistivity and alters capacitive behavior. Conversely, antioxidant treatment partially restores these electrical characteristics, which reflects improved membrane structure and function [28].

The dielectric response of liver tissue is closely related to the polarization of dipolar molecules within the membrane. Dielectric materials weaken the effective electric field by aligning internal dipoles opposite to the applied field, which then increases capacitance [29]. The alignment behavior of polar molecules before and after exposure to an external electric field is illustrated in Figure 6. The changes in the dielectric constant as observed in this study, therefore, reflect alterations in membrane composition and integrity at the molecular level.

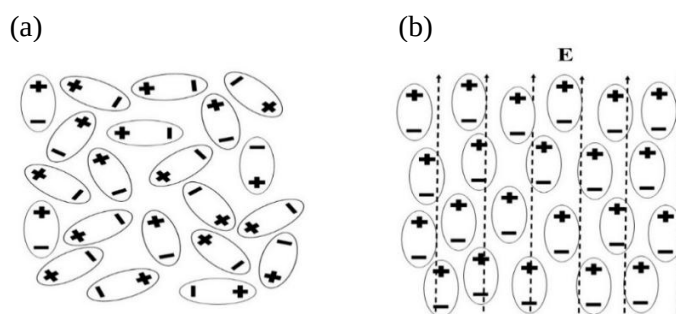


Figure 6 Polar molecules inside a dielectric material before receiving an external electric field (a) and after receiving an external electric field (b).

Damaged liver tissue exhibited lower resistivity due to increased membrane permeability that facilitates uncontrolled ion exchange between intra- and extracellular compartments. Histopathological findings strongly supported these electrical observations, where benzo[a]pyrene exposure induced chromatin condensation, nuclear fragmentation, cellular shrinkage, and eventual membrane rupture. The consistency between microscopic damage and electrical measurements strengthens the biological relevance of the bioelectrical approach that is used in this study, rather than positioning it as a purely technical assessment [30].

The present findings are consistent with previous reports demonstrating that antioxidant-mediated hepatoprotection is closely associated with preservation of membrane integrity and function. Studies on *Moringa oleifera* have shown reduced lipid peroxidation and improved hepatocyte morphology in oxidative stress models. At the same time, *Allium sativum* has been reported to stabilize membrane phospholipids and modulate ion transport systems [31]. Similarly, *Annona muricata* extracts have been shown to protect cellular membranes against oxidative injury. Recent investigations suggest that improvements in membrane integrity are reflected by normalization of the bioelectrical properties of tissue, such as increased resistivity and controlled dielectric behavior. The results of this study extend these findings by integrating histopathological evidence with bioelectrical measurements, which provides a complementary and non-destructive approach for the assessment of liver tissue recovery following exposure to toxins [32,33].

An important observation in this study is the reduced protective effect as observed at higher antioxidant doses, particularly in the moringa treatment groups, which suggests a potential transition from antioxidant to pro-oxidant activity. High concentrations of antioxidant compounds have been reported to promote oxidative stress by generating reactive intermediates, disrupting mitochondrial electron transport, or interfering with endogenous redox signaling pathways. This phenomenon, which is known as the antioxidant-pro-oxidant shift, has been documented for polyphenols, flavonoids, and sulfur-containing compounds under supraphysiological dosing conditions [34]. The decline in electrical recovery and the persistence of histological damage at higher doses in this study align with this mechanism; this underscores that antioxidant efficacy is dose-dependent and that excessive dosing may exacerbate cellular stress rather than confer protection. In simplified terms, healthy liver cells function as effective electrical barriers that limit ion movement as well as maintain high resistivity and stable dielectric properties. Benzo[a]pyrene-induced membrane damage weakens this barrier; this condition allows ions to move freely and results in reduced resistivity and altered capacitance. Antioxidant treatment partially restores membrane integrity and thereby improves electrical and histological indicators of liver health. Therefore, bioelectrical measurements in this study should be interpreted as functional reflections of membrane integrity and oxidative injury, rather than purely physical or abstract electrical phenomena [35].

5. Conclusion

This study demonstrates that antioxidant extracts from *Annona muricata*, *Allium sativum*, and *Moringa oleifera* exert significant hepatoprotective effects against benzo[a]pyrene-induced liver damage, with optimal protective responses being consistently observed at the third dose levels, as 3.9 mg/kg BW for *Annona muricata*, 2.3 mg/kg BW for *Allium sativum*, and 5.9 mg/kg BW for *Moringa oleifera*. Among the tested extracts, *Moringa oleifera* at 5.9 mg/kg BW shows the most prominent protective effect, as evidenced by histopathological improvement and restoration of liver electrical properties. An integrated evaluation of histopathological changes and bioelectrical parameters highlights membrane integrity as a shared mechanistic basis for hepatoprotection. It supports the use of electrical property measurements as complementary quantitative indicators of tissue recovery. For future experimental and translational perspectives, further studies are recommended to include pharmacokinetic-based dose optimization, long-term toxicity assessment, standardized extract formulation, and validation of bioelectrical measurements as potential non-invasive biomarkers in preclinical and clinical liver injury models.

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7. Author Contributions

U.P. Juswono: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. C.S. Widodo: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. J.A.E. Noor: Methodology, Resources, Supervision, Writing – review & editing. R. Latifah: Formal analysis, Validation, Visualization, Writing – review & editing. N.L. Sari: Data curation, Investigation, Visualization, Writing – original draft. M. Faisal: Data curation, Formal analysis, Investigation, Writing – original draft.

8. Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

9. References

- [1] Hedfi A, Ali M Ben, Hassan MM, Albogami B, Al-Zahrani SS, Mahmoudi E, et al. Nematode traits after separate and simultaneous exposure to Polycyclic Aromatic Hydrocarbons (anthracene, pyrene and benzo [a] pyrene) in closed and open microcosms. *Environ Pollut.* 2021;276:116759.
- [2] Porwisiak P, Werner M, Kryza M, Vieno M, Holland M, ApSimon H, et al. Modelling benzo (a) pyrene concentrations for different meteorological conditions—Analysis of lung cancer cases and associated economic costs. *Environ Int.* 2023;173:107863.
- [3] Chou YC, Lin YH, Lin PH, Tung YC, Ho CT, Pan MH. Dietary 5-demethylnobiletin modulates xenobiotic-metabolizing enzymes and ameliorates colon carcinogenesis in benzo [a] pyrene-induced mice. *Food Chem Toxicol.* 2021;155:112380.
- [4] Fan T, Xie Y, Ma W. Research progress on the protection and detoxification of phytochemicals against aflatoxin B1-Induced liver toxicity. *Toxicon.* 2021;195:58-68.
- [5] Song W, Wang Y, Huang T, Liu Y, Chen F, Chen Y, et al. T-2 toxin metabolism and its hepatotoxicity: New insights on the molecular mechanism and detoxification. *Environ Pollut.* 2023;330:121784.
- [6] Kesmati M, Tamoradi N, Rezaie A, Shahriyari A, Torabi M. Evaluating behavioral, biochemical and histopathological effects of the MgO nanoparticles administration on memory in the Alzheimer-like model of male rat. *Nanomedicine J.* 2021;8(2):140-146.
- [7] Erhonyota C, Edo GI, Onoharigho FO. Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. *Acta Ecol Sin.* 2023;43(4):684-689.
- [8] Vijay M, Rajitha M, Krishnagaanth M, Jadhav ND, Chigure G, Srivastava A. Controlling deltamethrin-resistant *Rhipicephalus microplus* with a phytoformulation of *Annona muricata* and *Piper longum*. *Acta Trop.* 2025;107644.
- [9] Faisal M, Juswono UP, Santoso DR, Widodo CS. The Effectiveness of Herbal Medicine Mangosteen Peel Extract (*Garcinia mangostana*) to Prevent Free Radicals Occurrence and Decrease in Hemoglobin Levels in the Blood Caused by Diabetes Mellitus. *Trends Sci.* 2025;22(3):9250.
- [10] Noor JAE, Juswono UP, Latifah R, Ada'Palentek G, Faisal M. Comparative analysis of herbs ability Bajakah wood (*Spatholobus littoralis*), red betel (*Piper crocatum*), and Papuan red fruit (*Pandanus conoideus*) in preventing free radicals due to rhodamine-B injection. In: *BIO Web of Conferences. EDP Sciences*; 2025. p. 2007.
- [11] Faisal M, Juswono UP, Santoso DR. The effect of extract guava, bitter melon, broccoli, and shallots on liver damage due to indoor pollutant exposure. In: *Journal of Physics: Conference Series. IOP Publishing*; 2021. p. 12097.
- [12] Faisal M, Juswono UP, Santoso DR. The dielectric properties of skin damage and its correlation to free radical intensity caused by UVA/UVB radiation impact. In: *Journal of Physics: Conference Series. IOP Publishing*; 2022. p. 12053.
- [13] Iskandar D, Widodo N, Warsito M, Rollando YPPA. Phenolic content, antioxidant, cytotoxic of fractions of *Spatholobus littoralis* Hassk from Kalimantan, Indonesia. *J Hunan Univ Nat Sci.* 2022;49(3).
- [14] Faisal M, Juswono UP, Santoso DR, Widodo CS. Analysis of diabetes mellitus screening method based on electrical properties of cells using dielectric method. In: *BIO Web of Conferences. EDP Sciences*; 2025. p. 2006.

- [15] Asgari-Kafrani A, Fazilati M, Nazem H. Hepatoprotective and antioxidant activity of aerial parts of *Moringa oleifera* in prevention of non-alcoholic fatty liver disease in Wistar rats. *South African J Bot.* 2020;129:82-90.
- [16] Badmus JA, Rafiu MA, Fatoki JO. The protective effect of ethanol leaf extract of *Annona muricata* against doxorubicin toxicity via modulations of hematological, serum biochemical, antioxidant enzymes, and lipid peroxidation. *Phytomedicine Plus.* 2022;2(3):100328.
- [17] Chan WJJ, McLachlan AJ, Hanrahan JR, Harnett JE. The safety and tolerability of *Annona muricata* leaf extract: a systematic review. *J Pharm Pharmacol.* 2020;72(1):1-16.
- [18] Tabbassum S, Cheng P, Yanko FM, Balachandran R, Aschner M, Bowman AB, et al. Whole body potassium as a biomarker for potassium uptake using a mouse model. *Sci Rep.* 2021;11(1):6385.
- [19] Bakonyi I. Accounting for the resistivity contribution of grain boundaries in metals: critical analysis of reported experimental and theoretical data for Ni and Cu. *Eur Phys J Plus.* 2021;136(4):410.
- [20] Aatresh AA, Alabhya K, Lal S, Kini J, Saxena PUP. LiverNet: efficient and robust deep learning model for automatic diagnosis of sub-types of liver hepatocellular carcinoma cancer from H&E stained liver histopathology images. *Int J Comput Assist Radiol Surg.* 2021;16(9):1549-1563.
- [21] Schreiber JB, Amin MEK. Core reporting expectations for quantitative manuscripts using independent and dependent t-tests, One-Way ANOVA, OLS regression, and Chi-Square. *Curr Pharm Teach Learn.* 2025;17(12):102462.
- [22] Elammari NE, Sariti SR. Anti-Leishmanial drug Pentostam induced histological changes to liver and kidney in male BALB/c wild mice. *Heal Sci.* 2021;1(2):7-14.
- [23] Burton L, Scaife P, Paine SW, Mellor HR, Abernethy L, Littlewood P, et al. Hydrostatic pressure regulates CYP1A2 expression in human hepatocytes via a mechanosensitive aryl hydrocarbon receptor-dependent pathway. *Am J Physiol Physiol.* 2020;318(5):C889-902.
- [24] Güvenç M, Cellat M, Gökçek İ, Özkan H, Arkalı G, Yakan A, et al. Nobiletin attenuates acetaminophen-induced hepatorenal toxicity in rats. *J Biochem Mol Toxicol.* 2020;34(2):e22427.
- [25] Zheng S, Huang L, Sun Z, Barboiu M. Self-assembled artificial ion-channels toward natural selection of functions. *Angew Chemie Int Ed.* 2021;60(2):566-597.
- [26] Ding T, Zhu J, Guan H, Xia D, Xing Y, Huang J, et al. Photothermally triggered melting and perfusion: responsive colloidosomes for cytosolic delivery of membrane-impermeable drugs in tumor therapy. *J Mater Chem B.* 2022;10(7):1103-1115.
- [27] Kumar A, Arora A, Sharma P, Jan S, Ara I. Visceral Fat and Diabetes: Associations With Liver Fibrosis in Metabolic Dysfunction–Associated Steatotic Liver Disease. *J Clin Exp Hepatol.* 2025;15(1):102378.
- [28] Song K, Lee E, Lee HS, Lee H, Kim JY, Choi Y, et al. Dual-energy X-ray absorptiometry and bioelectrical impedance analysis are useful for predicting metabolic syndrome and nonalcoholic fatty liver disease in children and adolescents. *Endocrine.* 2025;90(2):545-557.
- [29] Nishiwaki K, Saeki C, Kanai T, Sunayama M, Tamura Y, Niwa T, et al. Bioelectrical impedance analysis-derived phase angle predicts osteosarcopenia and mortality in patients with chronic liver disease. *Sci Rep.* 2025;15(1):24857.
- [30] Song K, Kwon YJ, Lee E, Lee HS, Youn YH, Baik SJ, et al. Bioelectrical impedance analysis parameters are superior to liver enzymes in predicting metabolic dysfunction-associated steatotic liver disease in young adults. *Intern Emerg Med.* 2025;1-11.
- [31] Okorie I, Nnam NM, Chukwuemeka EN, Goodluck C. Nutrient and Phytochemical Profile of Aqueous Extracts of Garlic (*Allium Sativum*) And Soursop (*Annona Muricata*) Leaves For Use As Hypoglycemic Agents. *World J Pharm Res.* 2023;12(5):1-17.
- [32] Dong J, Hu R, Xu X, Chen J, Niu Y, Wang F, et al. A facile in situ surface-functionalization approach to scalable laminated high-temperature polymer dielectrics with ultrahigh capacitive performance. *Adv Funct Mater.* 2021;31(32):2102644.
- [33] Mandurrino M, Arcidiacono R, Boscardin M, Cartiglia N, Dalla Betta GF, Ferrero M, et al. Analysis and numerical design of Resistive AC-Coupled Silicon Detectors (RSD) for 4D particle tracking. *Nucl Instruments Methods Phys Res Sect A Accel Spectrometers, Detect Assoc Equip.* 2020;959:163479.
- [34] Guneidy RA, Gad AM, Zaki ER, Ibrahim FM, Shokeer A. Antioxidant or pro-oxidant and glutathione transferase P1-1 inhibiting activities for *Tamarindus indica* seeds and their cytotoxic effect on MCF-7 cancer cell line. *J Genet Eng Biotechnol.* 2020;18(1):74.
- [35] Faisal M, Juswono UP, Santoso DR, Widodo CS. Correlation of electrical impedance values to blood sugar levels of mice (*Mus musculus*) with diabetes mellitus. In: *Journal of Physics: Conference Series.* IOP Publishing; 2025. p. 12003.