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# Comparative Evaluation of Hepatotoxic Effects of *Melia dubia* and *Andrographis paniculata* in Zebrafish Larvae

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## Abstract

Indian herbal medicines are extensively used in pharmaceutical, nutraceutical, food, cosmetic, and personal care industries for their therapeutic properties. However, Herb-Induced Liver Injury (HILI) has emerged as a significant global health concern, with several herbal preparations associated with hepatotoxicity. *Melia dubia* (MD) and *Andrographis paniculata* (AP) possess antimicrobial, antiviral, and anticancer activities, but their hepatotoxic effects remain poorly characterized. In this study, zebrafish (*Danio rerio*) embryos were used as an *in-vivo* vertebrate model for hepatotoxicity screening due to their conserved liver physiology and genetic similarity to humans. The 96 hpf $LC_{50}$ values of AP and MD were 555.19 ppm (0.5551 mg/mL) and 603.61ppm (0.6036 mg/mL), respectively. Developmental abnormalities, including bent spine, yolk sac oedema, pericardial oedema, were observed at concentrations ≥156.25 ppm for AP and ≥312.5 ppm for MD, while no deformities were detected at 156.25 ppm in the MD group. Both extracts significantly reduced heart rate compared with the control group (168 bpm). Liver biomarkers showed marked elevation, with ALT increasing by 196% in AP and 161% in MD, and AST increasing by 140% and 131%, respectively, at the highest tested concentrations. These findings confirm dose-dependent hepatotoxicity of both herbal extracts, with AP exhibiting greater toxic potential than MD.

**Key Findings:** AP and MD exhibited dose-dependent hepatotoxicity with $LC_{50}$ values of 0.5551 mg/mL (AP) and 0.6036 mg/mL (MD) at 96 hpf. Zebrafish embryos exhibited developmental abnormalities and significantly reduced heart rate. Elevated ALT (up to 196%) and AST (up to 140%) confirmed liver-specific toxicity; AP demonstrated greater hepatotoxic potential than MD.

**Keywords:** Developmental Deformities, Hepatotoxicity, $LC_{50}$ Value, Liver Biomarkers, Phytochemical Analysis, Zebrafish

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## 1. Introduction

Herbal medicines are formulations obtained from plant extracts with recognised therapeutic value, used across generations in traditional medicine systems worldwide. In India, approximately 70% of the rural population relies on the Ayurvedic medicine system for primary healthcare[1]. The global market for herbal supplements continues to expand rapidly, reflecting widespread consumer confidence in plant-based remedies. Plants such as ginger, walnuts, and green tea are valued for their medicinal properties, attributed to phytochemicals including alkaloids, phenolic compounds, flavonoids, and terpenoids that underlie their pharmacological activity[2]. However, contrary to popular belief, herbal medicines are not inherently safe. HILI is an increasingly recognised clinical entity that contributes substantially to Drug-Induced Liver Injury (DILI) worldwide. A comprehensive review of Indian Ayush herbal medicines published in 2024 reported that HILI prevalence among cirrhotic patients in India reached 63%, with mortality rates ranging from 10–19% in those without preexisting liver disease, rising to over 50% in those with underlying hepatic pathology[3]. A review of HILI cases published in

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2024 noted that herbal and dietary supplements are the second most common cause of DILI globally, accounting for approximately 25.3% of cases[4].

Despite their well-known therapeutic benefits, herbal medicines carry risks when used without proper dosage guidance, quality control, or awareness of drug interactions. The phytochemicals present in medicinal herbs exhibit a complex dual role: Many flavonoids, phenolics, alkaloids, and saponins exhibit hepatoprotective properties at physiological doses through antioxidant, anti-inflammatory, and free radical-scavenging mechanisms[5], yet these same compound classes can induce hepatocellular damage at high concentrations through mechanisms including Reactive Oxygen Species (ROS) generation, mitochondrial dysfunction, and disruption of bile acid transport[6]. A notable example is pyrrolizidine alkaloids found in various herbal remedies, which induce Hepato-Sinusoidal Obstruction Syndrome (HSOS) through oxidative damage to hepatic sinusoidal endothelial cells[4]. Similarly, aristolochic acid from *Aristolochia fangchi* is associated with nephrotoxicity and carcinogenicity[7]. Therefore, a comprehensive toxicological evaluation of medicinal herbs is essential before widespread or chronic therapeutic use.

*Melia dubia* (MD), a tree belonging to the family Meliaceae, is distributed throughout India, Sri Lanka, and Southeast Asia, and is commonly found in forests and agroforestry systems. Its leaves, flowers, seeds, oil, root, young branches, fruits, and bark possess recognised medicinal benefits[8] Historically, extracts of different parts of the MD plant exhibit anticancer, anti-inflammatory, antioxidant, and antimicrobial activities[9,10]. Gas Chromatography- Mass Spectrometry (GC-MS) analysis of *M. dubia* leaf extracts has identified 42 phytochemicals, including terpenoids (caryophyllene, humulene, germacrene-D), unsaturated fatty acids (octadecanoic acid, hexadecanoic acid), phenolic derivatives, and lipophilic organic compounds with documented insecticidal, anti-inflammatory, antioxidant, and antibacterial properties[11]. Members of the genus *Melia* are also notable for their content of limonoids—highly oxygenated triterpene derivatives—which exhibit broad-spectrum antiviral, antifungal, anti-inflammatory, and cytotoxic activities[12]. Recent studies have demonstrated that *Melia azedarach* (a closely related species) exhibits anti-SARS-CoV-2 activity with an $IC_{50}$ of 6.922 µg/mL, attributed to its flavonoid and phenolic enrichment[13]. However, extracts of MD bark, leaves, and fruits have been reported to cause severe adverse effects, including neurological, cardiovascular, gastrointestinal effects, and death in severe cases[14]. Despite its widespread traditional use, the hepatotoxic potential of MD at sub-lethal concentrations has not been systematically evaluated in any standardised animal model, and no 96 hpf $LC_{50}$ value has been previously reported.

*Andrographis paniculata* (AP), commonly known as 'King of Bitters' (*Kalmegh*), belongs to the family Acanthaceae and is predominantly found in South India, Sri Lanka, and Southeast Asia. AP leaves and roots have been used in Ayurveda, Traditional Chinese Medicine (TCM), and Thai traditional medicine to treat fever, hypertension, diarrhoea, malaria, diabetes[15], and snake bite[16]. The pharmacological activities of AP are primarily attributed to its major diterpenoid lactone andrographolide, which accounts for approximately 0.5–6% of dry leaf weight and exhibits antimicrobial, anti-inflammatory, antiviral, and immunostimulatory properties[17]. A 2023 systematic review confirmed that AP is clinically effective for acute uncomplicated respiratory tract infections, reducing symptom duration and supporting immune function[18]. Both AP and MD are thus employed in overlapping therapeutic contexts, particularly for fever, infections, and general immune support—contexts where they are frequently co-prescribed or self-administered in Indian traditional medicine.

Despite AP's broad therapeutic use, clinical evidence of its hepatotoxic potential has emerged. During the COVID-19 pandemic in Thailand (2019–2021), AP was recommended as a supplementary treatment; A retrospective cohort study demonstrated that patients using AP had significantly elevated ALT levels compared to non-users, suggesting hepatocellular damage[19]. Furthermore, a 2024 retrospective study evaluating the combined use of AP and favipiravir in COVID-19 patients reported adverse effects on liver biochemistry parameters, underscoring the importance of monitoring liver function during AP use[20]. Experimental studies in rats have shown that the methanolic extract of AP and its active constituent, andrographolide, significantly altered serum AST, ALT, ALP, and bilirubin levels when administered at high doses in Carbon Tetrachloride ($CCl_4$-induced) hepatotoxicity models, demonstrating a complex and dose-dependent dual role—hepatoprotective at low doses, potentially hepatotoxic at high concentrations[21]. This dose-dependency highlights the critical importance of establishing safe concentration thresholds for AP use.

Zebrafish (*Danio rerio*) have emerged as a powerful vertebrate model for hepatotoxicity research. Their high genomic similarity to humans (>87% gene homology; ~80% of human disease-associated genes have zebrafish orthologs), external fertilisation, optical transparency, and rapid development make them ideal for high-throughput *in vivo* toxicity screening[22]. Recent reviews have recognised Zebrafish as an indispensable tool in drug discovery and hepatotoxicity pharmacology, highlighting their ability to provide *in vivo* information equivalent to mammalian studies at a fraction of the cost[23]. The zebrafish liver is functional by 72hpf and shares structural and functional homology with the mammalian liver, including conserved bile acid transport pathways, cytochrome P450-based drug metabolism, and lipid homeostasis mechanisms—all of which have been exploited for DILI modelling[24]. Multiple recent studies have validated the zebrafish as a reliable model for detecting herbal hepatotoxicity: ALT and AST elevation after exposure to *Psoralea corylifolia* aqueous extract was confirmed in zebrafish in 2024[25], and similar hepatotoxic responses were reported for *Rhizoma Paridis*, toosendanin (from *Melia toosendan*), and triptolide (from *Tripterygium wilfordii*) in the same model system[26,27]. Previous studies on MD have focused on antimicrobial and cytotoxic properties. No study has yet reported the 96hpf $LC_{50}$ of MD in zebrafish. Similarly, while AP toxicity has been partially evaluated in zebrafish, a direct comparative hepatotoxicity assessment using liver-specific biomarkers is lacking. The present study addresses this research gap by providing a comprehensive comparative evaluation of the hepatotoxic effects of AP and MD using liver biomarkers (ALT and AST), developmental endpoints, and acute toxicity parameters. The study hypothesises that both AP and MD induce dose-dependent hepatotoxicity in zebrafish, with AP exhibiting greater hepatotoxic potential based on available clinical evidence.

# 2. Materials and Methods

## 2.1 Preparation of *Melia dubia* (MD) and *Andrographis paniculata* (AP) Leaf Extract

The leaf powder of MD and AP was purchased from a certified Siddha medicine shop in Tambaram Sanatorium, Chennai, Tamil Nadu, India. Aqueous extraction was performed by dissolving 5g of leaf powder in 150mL of distilled water and heating at 60°C for 1 hour. The extract was filtered through muslin cloth and centrifuged at 3,000 × g for 30 minutes to remove fine particles. The supernatant was stored at 4°C until use. For $LC_{50}$ determination, the infusion method was used: 1 g of leaf powder was boiled in 50mL of E3 embryo medium for 10 minutes, then filtered to obtain the stock extract at a concentration of 5000ppm.

## 2.2 Phytochemical Analysis of Leaf Extracts

Qualitative phytochemical screening of the crude aqueous extracts of MD and AP was performed to identify the major secondary metabolite classes using standard protocols[28]. Identification of phytochemicals is essential to provide a mechanistic basis for the observed biological activities, as constituents such as flavonoids, alkaloids, tannins, and saponins are known to exhibit both hepatoprotective and hepatotoxic properties depending on concentration[5]. The tests were performed as described in Table 1.

## 2.3 Maintenance of Zebrafish

Adult Zebrafish (wild type) were purchased from a Committee for Control and Supervision of Experiments on Animals (CCSEA) approved vendor and acclimatised for one month to reach reproductive maturity. The

**Table 1.** Qualitative phytochemical screening tests of crude extract

| S. No | Phytochemical | Test Performed | Procedure |
|---|---|---|---|
| 1 | Proteins and Amino Acids | Ninhydrin Test | 2 mL extract + 2 mL 0.2% Ninhydrin, boiled |
| 2 | Carbohydrates | Benedict's Test | 2 mL extract + 2 mL Benedict's reagent, boiled |
| 3 | Phenols and Tannins | Ferric Chloride Test | 2 mL extract + 2 mL 2% $FeCl_3$ |
| 4 | Flavonoids | Alkaline Reagent Test | 2 mL extract + 2 mL 2% NaOH, then diluted with acid |
| 5 | Saponins | Foam Test | 2 mL extract + 5 mL distilled water, shaken |
| 6 | Cardiac Glycosides | Keller-Killiani Test | 2 mL extract + glacial acetic acid + $FeCl_3$, layered with $H_2SO_4$ |
| 7 | Alkaloids | Mayer's Test | 2 mL extract + 1% HCl, heated, then Mayer's reagent |

study was approved by the Institutional Animal Ethics Committee, Sri Venkateswara College of Engineering (Approval No: SVCE/BIO/IAEC/02-2023/01). Male and female zebrafish were maintained separately (Figure 1). The fish were maintained at pH 7.0 and 25–27°C under a 14:10 light: dark photoperiod.

## 2.4 Breeding of Zebrafish and Collection of Embryos

Zebrafish were placed for breeding in a 2:1 female-to-male ratio in the evening, and embryos were collected the following morning, ensuring equal light and dark exposure. Embryos were collected using three-stage strainers, washed with distilled water, and maintained in E3 embryo medium until use. Only fertilized, morphologically normal embryos at the 4–8 cell stage were selected for exposure experiments.

## 2.5 Determination of $LC_{50}$ of Herbal Medicine Extracts

To determine the median lethal concentration ($LC_{50}$) of MD and AP, fertilized embryos were randomly distributed into 6-well plates with 10 embryos per well (n = 30 per concentration group, 3 replicates). Embryos were exposed to six concentrations (5000, 2500, 1250, 625, 312.5, and 156.25ppm) of each extract from 4hpf to 96hpf. A vehicle control group (E3 medium only) was maintained in parallel. Exposure solutions were renewed every 24 hours. Dead or coagulated embryos were removed and recorded daily. Mortality endpoints included: Coagulation of the embryo, lack of somite formation, non-detachment of the tail, and absence of heartbeat. Probit regression analysis was performed using SPSS software to determine the 96hpf $LC_{50}$ values[29].

## 2.6 Hatching Rate and Heart Rate

Hatching rate was monitored at 48hpf and 72hpf following the Organization for Economic Cooperation and Development Guidelines for the Testing of Chemicals (OECD) Test No. 236 guidelines[29]. Heart rate was measured by mounting individual larvae on a glass slide and counting heartbeats for 60 seconds under a light microscope at 10× magnification. Statistical significance was assessed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

## 2.7 Liver Function Assay

After 96hpf exposure to various concentrations of MD and AP, 80 larvae per concentration group were pooled and homogenised in cold saline (w/v = 1:9). Homogenates were centrifuged at 2,500 rpm for 10 minutes, and supernatants were collected for enzymatic analysis[30]. ALT activity was determined using the colourimetric method of Reitman and Frankel (1957), in which ALT catalyses the transamination of alanine and α-ketoglutarate to produce pyruvate, which

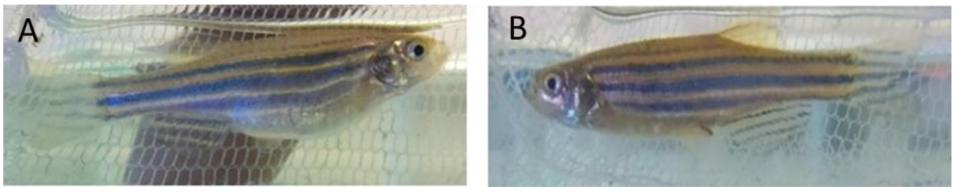



**Figure 1.** *Danio rerio*: (A) Female zebrafish, (B) Male zebrafish.

**Table 2.** Qualitative phytochemical screening results of MD and AP extracts

| S. No | Test Performed | Observation | Inference |
|---|---|---|---|
| 1 | Ninhydrin Test | Violet Colour | Presence of proteins and amino acids |
| 2 | Benedict's Test | Reddish-Brown Precipitate | Presence of carbohydrates |
| 3 | Ferric Chloride Test | Blue-green or black coloration | Presence of phenols and tannins |
| 4 | Alkaline Reagent Test | Intense yellow → Colourless after acid addition | Presence of flavonoids |
| 5 | Foam Test | Stable Foam Formation | Presence of saponins |
| 6 | Keller-Killiani Test | Brown ring at the interphase | Presence of cardiac glycosides |
| 7 | Mayer's Test | White creamy or reddish-brown precipitate | Presence of alkaloids |

reacts with 2,4-dinitrophenylhydrazine to form a colored hydrazone complex measured at 505nm. AST activity was measured by the same colourimetric principle, measuring the transamination of aspartate and α-ketoglutarate to oxaloacetate, measured at 505nm[31]. Results were expressed as IU per gram of protein.

# 3. Results

## 3.1 Qualitative Phytochemical Screening of Crude Extract

Aqueous extraction of *Melia dubia* and *Andrographis paniculata* leaf powders was performed, and the extracts were subjected to standard qualitative phytochemical screening. Both extracts were found to contain proteins and amino acids, carbohydrates, phenols and tannins, flavonoids, saponins, cardiac glycosides, and alkaloids (Table 2). The presence of alkaloids and flavonoids in both extracts is of particular hepatotoxicological relevance. At high concentrations, alkaloids can undergo cytochrome P450-mediated bioactivation, forming reactive metabolites that covalently bind hepatocellular proteins, triggering oxidative stress and mitochondrial dysfunction[6]. Flavonoids, while hepatoprotective at physiological doses through Nrf2 pathway activation and free radical scavenging[5], have been reported to induce pro-oxidant activity at supra-physiological concentrations, contributing to ROS-mediated hepatocellular damage[34]. Similarly, saponins can perturb membrane integrity and increase hepatocyte permeability, facilitating the release of intracellular enzymes such as ALT and AST into circulation—a proposed mechanism for the elevated transaminase levels observed in the present study[33].

## 3.2 Determination of $LC_{50}$ Values

The $LC_{50}$ values of MD and AP extracts were determined at 96 hpf by probit regression analysis. The $LC_{50}$ of MD was 603.61 ppm (0.6036 mg/mL), and that of AP was 555.19 ppm (0.5551 mg/mL), indicating that AP is more acutely toxic than MD to zebrafish embryos. The mortality data are summarised in Tables 3 and 4, and probit curves are shown in Figure 2.

## 3.3 Hatching Rate of Embryos

The toxicity of MD and AP affects embryo hatching. Delayed hatching was observed at the lowest concentrations of both MD and AP (Figure 3), but all the embryos hatched at 96 hpf (Figure 4). In the control group, hatching commenced at 48 hpf and was complete by 72 hpf.

## 3.4 Heart Rate of Larvae

Heart rate exhibited a significant, concentration-dependent decrease in both MD and AP exposure groups relative to the control group (168 ± 2.08bpm; $p < 0.05$, one-way ANOVA). In the AP group, heart rates recorded were 132 ± 1.87bpm (625ppm), 141 ± 2.13bpm (312.5ppm), and 154 ± 1.60 bpm (156.25ppm). For MD, heart rates were 143 ± 1.45bpm (625ppm), 152 ± 1.92bpm (312.5ppm), and 160 ± 1.29bpm (156.25ppm). AP caused a greater reduction in heart rate than MD at equivalent concentrations (Table 5).

## 3.5 Developmental Deformities at 96 hpf

As shown in Figure 5, various types of malformations were observed in the embryonic-larval stages of zebrafish exposed to MD and AP, such as Bent Spine (BS), Pericardial

**Table 3.** Mortality of embryos exposed to various concentrations of MD extract

| Dose (ppm) | Total Embryos | Dead Embryos (Mean ± SE) | Log Dose |
|---|---|---|---|
| 0 (Control) | 30 | 0 ± 0.00 | – |
| 156.25 | 30 | 0 ± 0.00 | 2.194 |
| 312.5 | 30 | 4 ± 0.58 | 2.495 |
| 625 | 30 | 17 ± 1.20 | 2.796 |
| 1250 | 30 | 26 ± 0.88 | 3.097 |
| 2500 | 30 | 30 ± 0.00 | 3.398 |
| 5000 | 30 | 30 ± 0.00 | 3.699 |

**Table 4.** Mortality of embryos exposed to various concentrations of AP extract

| Dose (ppm) | Total Embryos | Dead Embryos (Mean ± SE) | Log Dose |
|---|---|---|---|
| 0 (Control) | 30 | 0 ± 0.00 | – |
| 156.25 | 30 | 0 ± 0.00 | 2.194 |
| 312.5 | 30 | 10 ± 1.15 | 2.495 |
| 625 | 30 | 15 ± 0.88 | 2.796 |
| 1250 | 30 | 23 ± 1.20 | 3.097 |
| 2500 | 30 | 30 ± 0.00 | 3.398 |
| 5000 | 30 | 30 ± 0.00 | 3.699 |

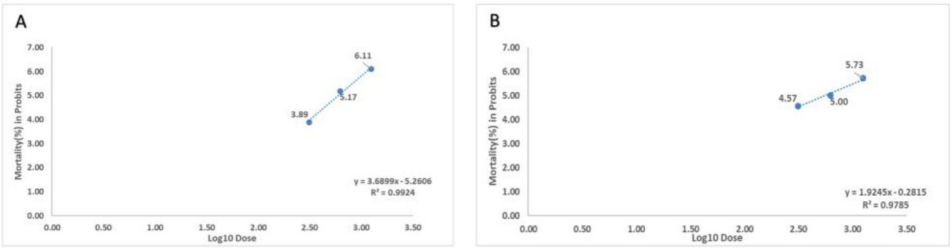



**Figure 2.** Graph showing mortality (%) in probits vs $Log_{10}$ dose of (A) *Melia dubia* extract (B) *Andrographis paniculata* extract.

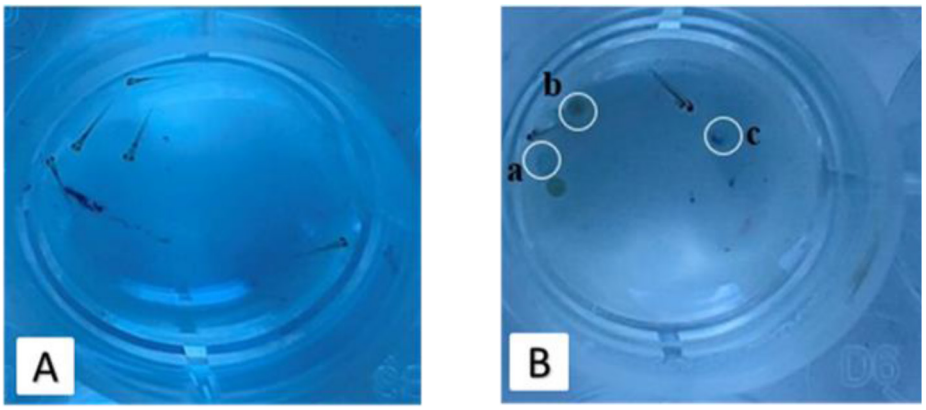



**Figure 3.** Hatching rate: (A) Embryos hatched in the control group, (B) Embryos hatched in the exposure group.

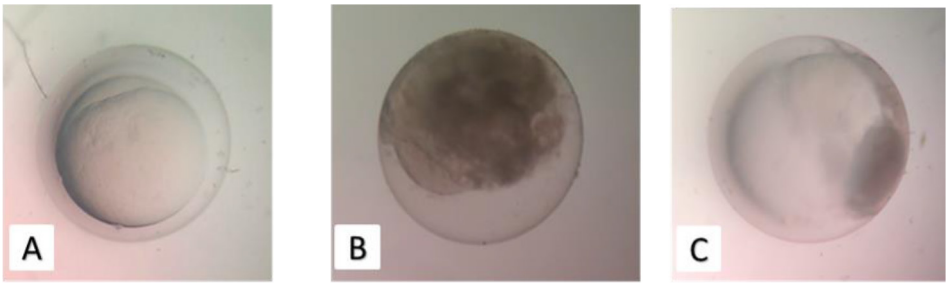



**Figure 4.** Embryos: (A) Unhatched healthy embryo, (B) Coagulated embryo – Dead, (C) Partially coagulated embryo under 10× magnification.

**Table 5.** Heart rate of Zebrafish larvae exposed to various concentrations of *Melia dubia* and *Andrographis paniculata* extracts (beats per minute, mean ± SE)

| Group | 625 ppm | 312.5 ppm | 156.25 ppm |
|---|---|---|---|
| MD | 143 ± 1.45* | 152 ± 1.92* | 16w0 ± 1.29* |
| AP | 132 ± 1.87* | 141 ± 2.13* | 154 ± 1.60* |
| Control | 168 ± 2.08 | 168 ± 2.08 | 168 ± 2.08 |

*$p < 0.05$ vs. control (one-way ANOVA, Tukey's post-hoc test)

Edema (PE), and Yolk Sac Edema (YSE). The percentage of malformations was higher in embryos exposed to AP, and malformations were observed in all larvae exposed to 625ppm, 312.5ppm, and 156.25ppm of AP. Whereas the malformation rate was significantly reduced in embryos exposed to MD, no malformations were observed at the lowest MD concentration (156.25ppm) or in the control group.

## 3.6 Liver Function Assay

The ALT and AST are the most commonly used biomarkers to assess the extent of hepatotoxicity caused by toxic compounds. ALT and AST activities were significantly higher than in the control. The results of ALT quantification showed a 161% increase in ALT

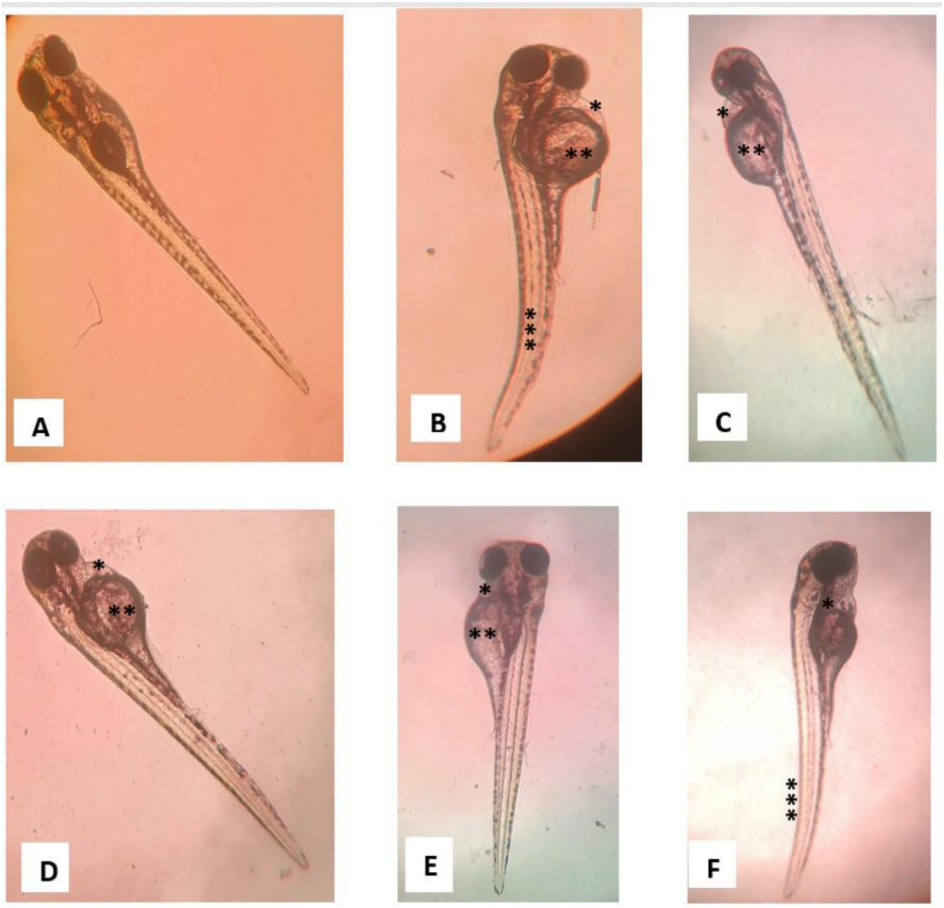



**Figure 5.** Larval malformations: (A) Control larvae, (B) Larvae in 625 ppm AP, (C) Larvae in 312.5 ppm AP, (D) Larvae in 156.25 ppm AP, (E) Larvae in 625 ppm MD, (F) Larvae in 312.5 ppm MD. *Pericardial edema, **Yolk sac edema, ***Bent spine.

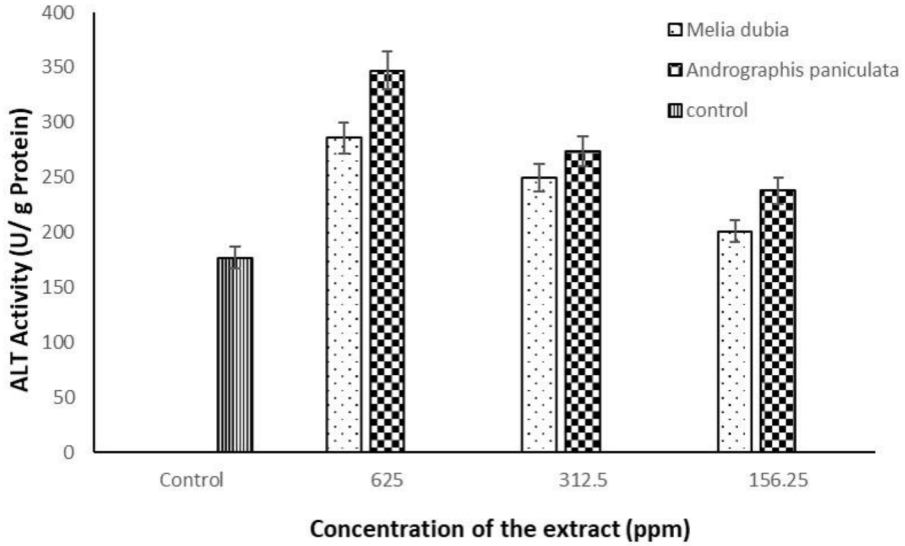



**Figure 6.** ALT activity (IU/g protein) in zebrafish larvae exposed to *Melia dubia* and *Andrographis paniculata* extracts. Values represent mean ± SE (n = 3). *$p < 0.05$ vs. control.

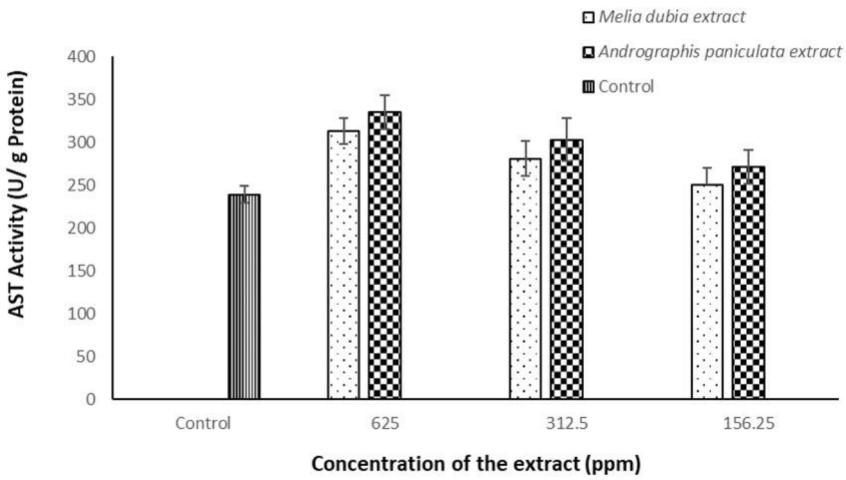



**Figure 7.** AST activity (IU/g protein) in zebrafish larvae exposed to *Melia dubia* and *Andrographis paniculata* extracts. Values represent mean ± SE (n = 3). *p < 0.05 vs. control.

activity in the higher concentration MD group and a 196% increase in the higher concentration AP group compared to the control (Figure 6). Similarly, AST quantification showed a 131% increase in AST activity in the higher concentration MD group and a 140% increase in the higher concentration AP group compared to the control (Figure 7). All elevations were dose-dependent and statistically significant ($p < 0.05$; one-way ANOVA, Tukey's post hoc test).

## 4. Discussion

Herbal medicines are deeply embedded in Indian healthcare practice, with approximately 70% of the rural population depending on Ayurvedic formulations[1]. Despite widespread use, HILI represents a growing clinical concern globally, and a 2024 review established that liver injury from Ayush formulations can cause acute-on-chronic liver failure with >50% fatality in patients with preexisting liver disease[3]. The increasing prevalence of HILI underscores the urgent need for systematic preclinical safety profiling of commonly used medicinal herbs, particularly those used chronically or at high doses[4].

Zebrafish represent a validated and widely accepted model for hepatotoxicity screening. With >87% genomic homology to humans, functional hepatic drug metabolism pathways including conserved CYP450 enzymes, and transparent larvae that permit direct visualisation of liver morphology, zebrafish are well-suited for high-throughput assessment of herb-induced liver injury[22,23]. The zebrafish liver is functional by 72 hpf and expresses conserved bile acid transport genes, lipid metabolism regulators (PPARα, FXR), and antioxidant enzymes (SOD, CAT, GSH), all central to liver injury responses[24]. Recent studies validating the zebrafish hepatotoxicity model include: elevated ALT and AST in zebrafish exposed to *P. corylifolia* aqueous extract with concomitant bile acid and lipid metabolism disturbances (2024)[25]; hepatocellular damage and elevated transaminases after toosendanin (from the related species *M. toosendan*) exposure through inflammation, autophagy, and apoptosis pathways[26]; and elevated ALT/AST with histological liver damage after triptolide exposure, exacerbated by inflammatory co-treatment[27]. These published models provide direct comparators for our results and collectively support the reliability of ALT and AST measurement in zebrafish as a liver-specific endpoint that translates to mammalian hepatotoxicity.

The phytochemical composition of both MD and AP extracts detected in the present study—alkaloids, flavonoids, tannins, saponins, and cardiac glycosides—provides a mechanistic basis for the observed hepatotoxic effects. The relationship between phytochemicals and liver injury is well established in the literature. Alkaloids, when present at high concentrations, undergo hepatic CYP450-mediated bioactivation to reactive electrophilic intermediates that deplete Glutathione (GSH), form protein adducts, and initiate mitochondrial dysfunction[6]. This mechanism mirrors the hepatotoxicity of pyrrolizidine alkaloids, which cause HSOS through selective toxicity to hepatic sinusoidal endothelial cells[4]. Tannins at high doses can precipitate intracellular

proteins and disrupt mitochondrial membrane potential, a precursor to the mitochondrial dysfunction and elevated ROS observed in herb-induced hepatotoxicity[34]. Saponins can perturb hepatocellular membrane integrity by forming complexes with membrane sterols, increasing permeability, and facilitating the release of intracellular enzymes such as ALT and AST[33]. The parallel positive detection of all major phytochemical classes in both MD and AP extracts suggests that multiple compound classes may act synergistically to produce the observed hepatotoxic effects, a possibility consistent with the dose-dependent elevation of ALT and AST measured in the present study.

A critical distinction must be drawn between the general toxic effects of MD and AP exposure (delayed hatching, reduced heart rate, developmental deformities) and the liver-specific effects evidenced by ALT and AST elevation. The developmental deformities and bradycardia observed at sub-lethal concentrations reflect systemic toxicity, likely mediated by oxidative stress and mitochondrial dysfunction affecting multiple organ systems[35]. In contrast, the elevation of ALT and AST specifically reflects hepatocellular damage. ALT, primarily localised in hepatocytes, is regarded as the most sensitive and specific biomarker of liver cell injury; its serum elevation is widely used as a diagnostic criterion for DILI in clinical settings (ALT ≥3× the upper limit of normal indicates hepatocellular injury)[36]. In zebrafish, as in mammals, hepatocyte damage leads to increased cell membrane permeability and release of ALT and AST into the circulation, providing a direct biochemical indicator of hepatocellular injury that has been validated in multiple published zebrafish DILI studies[24]. The significantly greater elevation of ALT (196%) compared to AST (140%) in AP-exposed groups at the highest concentration supports hepatocellular injury as the primary injury pattern, consistent with the higher hepatocyte-specificity of ALT. By contrast, the relatively smaller differential between ALT and AST increases in MD-exposed groups may reflect a mixed hepatocellular and mitochondrial injury pattern, since AST is enriched in mitochondria[37].

The delayed hatching observed at sub-lethal concentrations suggests that AP and MD exposure disrupts the enzymatic degradation of the chorion membrane mediated by choriolysin. This finding represents a general developmental toxicity endpoint rather than a liver-specific effect. Similar hatching delays at sub-lethal concentrations have been reported for other phytochemical exposures, including curcumin[38] and allicin from garlic[39]—common dietary plants in South India—suggesting a shared mechanism involving oxidative interference with chorionase activity.

The concentration-dependent reduction in heart rate observed in both AP and MD exposure groups indicates cardiotoxic effects at higher doses—a general rather than liver-specific finding. The decreased heart rate may be due to the generation of reactive oxygen species during embryogenesis and less contraction of the heart muscles. This proposed mechanism—supported by published literature but not directly measured in the current study—is consistent with reports that andrographolide modulates mitochondrial membrane potential and induces oxidative stress at high concentrations in experimental models[40]. These cardiotoxic effects should be considered as distinct from the hepatocellular damage evidenced by the liver enzyme elevations described above. Zebrafish is a well-established model for cardiovascular toxicity assessment due to its transparent embryos and conserved cardiac developmental pathways[24].

The acute toxicity test showed that the $LC_{50}$ for AP is 0.5551 mg/mL, consistent with a previously reported value of 0.5256 mg/mL[41]. This difference may be due to the locality of the leaf powder collected and the extraction process employed. Notably, this is the first study to report the 96 hpf $LC_{50}$ of MD in zebrafish (603.61ppm; 0.6036 mg/mL), providing a new benchmark for future comparative toxicological studies.

Developmental defects such as BS, YSE, and PE were observed in the embryos exposed to AP and MD extracts in a dose-dependent manner. Deformities were observed in all larvae in the AP exposure groups, whereas in embryos exposed to MD, deformities were limited to 625ppm and 312.5ppm. These deformities, consistent with those reported for turmeric extract[38] and garlic-derived compounds[39] in zebrafish, are proposed to arise from elevated ROS and mitochondrial dysfunction—a mechanism supported by multiple published studies but not directly measured in the current work. Elevated oxidative stress levels may be a contributing factor to the deformities observed in the MD and AP exposure groups. This is analogous to the aclonifen-mediated toxicity model in zebrafish, in which mitochondrial ETC inhibition and elevated oxidative stress led to diverse developmental abnormalities[35].

ALT and AST are vital aminotransferase enzymes widely used to evaluate liver cell damage. In a healthy person, the normal ranges for ALT and AST are 0–45

IU/L and 0–35 IU/L, respectively[43]. The results of ALT and AST activity show that the ALT and AST levels were found to be higher compared to the control group, confirming hepatocellular injury. The ALT and AST elevations observed (up to 196% for ALT, 140% for AST at the highest AP concentration) are comparable in magnitude to those reported in other zebrafish herbal hepatotoxicity studies. Gao *et al*. reported significant ALT and AST elevation in zebrafish exposed to *P. corylifolia* aqueous extract, accompanied by disrupted FXR/PPARα gene expression affecting bile acid and lipid metabolism[25]. Sun *et al*. demonstrated that toosendanin from the related species *M. toosendan* triggered ALT/AST elevation through inflammation, autophagy, and apoptosis pathways in zebrafish[26]. Li *et al*. further showed that triptolide-induced transaminase elevation was exacerbated by inflammatory co-treatment, highlighting the role of oxidative and inflammatory pathways in herbal hepatotoxicity[27]. Similar work carried out using *Euphorbia kansui* extract exposure to zebrafish embryos showed that there was increased ALT and AST levels, along with the loss of cellular architecture of the liver[30]. These findings support the notion that oxidative stress plays a central role in liver toxicity induced by both synthetic chemicals and certain herbal agents. These results also suggest that oxidative stress might be one of the factors in herbal medicine hepatotoxicity, as there are several studies that report the alteration in oxidative stress biomarkers on exposure to tricyclazole[42], isoniazid[43], and tebuconazole[44]. However, direct confirmation through measurement of ROS, Malondialdehyde (MDA), Superoxide Dismutase (SOD), Catalase (CAT), and Reduced Glutathione (GSH) levels would be required to conclusively establish this mechanistic pathway in future studies.

## 5. Conclusion

This is the first study to report the 96 hpf $LC_{50}$ value for *Melia dubia*, which was determined to be 0.6036 mg/mL. The 96 hpf $LC_{50}$ value of AP was determined to be 0.5551 mg/mL. The study compared the toxic effects of AP and MD by validating several parameters, including heart rate, hatch rate, embryo malformations, and liver biomarkers (ALT and AST). A clear distinction is established between general developmental toxicity effects (delayed hatching, bradycardia, morphological deformities) and liver-specific hepatocellular damage (elevated ALT and AST). The hatch rate in both exposure groups was delayed compared to the control group, even though all embryos hatched by 96 hpf. The average heart rate of both exposure groups was reduced compared to the control group. The developmental deformities were greater in embryos exposed to AP than in those exposed to MD, but none were observed in the control groups. The liver biomarkers were elevated in both exposure groups compared to the control. To clarify the mechanism of hepatotoxicity, gene expression profiling and direct measurement of oxidative stress biomarkers (ROS, MDA, SOD, CAT, GSH) should be carried out in future studies. The phytochemical constituents detected in both extracts—alkaloids, flavonoids, tannins, and saponins—are proposed to contribute to hepatotoxicity by inducing ROS-mediated mitochondrial dysfunction and hepatocellular membrane disruption, consistent with the published literature. However, MD is less toxic than AP, and using the extracts as a daily tonic may have detrimental consequences for human health; thus, these phytoextracts should be used with caution.

## 6. References


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Conceptualisation, S.P.P. and C.K.; methodology, S.P.P. and S.D.; validation, S.P.P. and S.D.; formal analysis, S.P.P. and S.D.; investigation, S.D.; resources, S.P.P. and S.D.; data curation, S.P.P. and S.D.; writing—original draft preparation, S.D.; writing—review and editing, S.P.P., S.D., and C.K.; visualisation, S.P.P., C.K., and S.D.; supervision, S.P.P. All authors have read and agreed to the published version of the manuscript.


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