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Protective effects of *Momordica charantia* and *Ocimum basilicum* extracts against azoxymethane-induced organ toxicity in rats

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Abstract. *Abisoye SB, Ezima EN, Adegbesan BO, Okelola CA, Bello TH, Adefuye AO. 2025. Protective effects of* Momordica charantia *and* Ocimum basilicum *extracts against azoxymethane-induced organ toxicity in rats. Asian J Trop Biotechnol 22: 71-79.* Azoxymethane (AOM) is a chemical carcinogen widely used in experimental models and is increasingly recognized for its systemic and extra-colonic toxic effects. This study evaluated the preclinical safety profile and organ-level biological effects of a combined aqueous extract of *Momordica charantia* and *Ocimum basilicum* against AOM-induced toxicity in Wistar rats. Acute toxicity was assessed using Lorke's method, followed by an experimental protocol in which rats were exposed to AOM and treated with graded doses of the combined extract for 28 days. Liver function biomarkers, including aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase, were analyzed, and histopathological examinations of the liver, kidney, and heart were conducted. Acute toxicity testing revealed no mortality at doses up to 5000 mg/kg, indicating a wide safety margin for the combined extract. AOM administration resulted in marked alterations in liver biochemical parameters and pronounced histological damage across examined organs. Treatment with the combined extract was associated with dose-related modulation of liver enzyme levels and descriptive preservation of hepatic, renal, and cardiac tissue architecture compared with the AOM-only group. The liver exhibited the most consistent response across biochemical and histological assessments, followed by the kidney and heart. Overall, the findings provide preclinical evidence supporting the biological relevance of the combined extract in mitigating AOM-induced systemic organ injury. This study provides preliminary evidence supporting the potential of these tropical plants as candidates for further phytochemical characterization and mechanistic investigation, while emphasizing their role as screening-level agents rather than confirmed therapeutic interventions.

Keywords: Azoxymethane, histopathology, *Momordica charantia, Ocimum basilicum*, tropical biotechnology

INTRODUCTION

Azoxymethane (AOM) is a well-established chemical carcinogen widely used in experimental models to study colorectal carcinogenesis; however, its biological effects extend beyond the colon and involve systemic toxicity (Bissahoyo et al. 2005; Stastna et al. 2019). Following administration, AOM undergoes hepatic and intestinal biotransformation by cytochrome P450 enzymes, producing highly reactive metabolites such as methylazoxymethanol that circulate systemically (Megaraj et al. 2014). These metabolites induce oxidative stress, DNA damage, and cellular dysfunction not only in colonic tissue but also in multiple extra-colonic organs, positioning AOM as a multisystem toxicological model rather than a colon-restricted one (Srisajjakul et al. 2022). Accordingly, AOM exposure provides a relevant experimental platform for evaluating systemic organ injury and for screening candidate agents with potential protective effects against chemically induced toxicity.

Among extra-colonic targets, the liver, kidney, and heart are particularly vulnerable to AOM-induced toxicity due to their central roles in metabolism, detoxification, and systemic circulation. Hepatic injury occurs as a direct consequence of AOM bioactivation, leading to hepatocellular damage, altered liver enzyme activity, and impaired protein synthesis (Matkowskyj et al.1999; Lima et al. 2019). Renal toxicity has been associated with the excretion of reactive metabolites and sustained oxidative stress, resulting in glomerular and tubular degeneration (Erseckin et al. 2022; Waggie et al. 2022). In addition, systemic inflammation and redox imbalance may compromise cardiac tissue integrity, contributing to myocardial distortion and inflammatory infiltration (Sonowal et al. 2017; Zanfirescu et al. 2019). These organ-specific manifestations underscore the importance of multi-organ assessment when evaluating both carcinogen-induced toxicity and the biological performance of candidate protective agents.

Tropical medicinal plants represent a rich source of bioactive compounds with diverse pharmacological properties and remain central to early-stage biotechnological research (Bishayee et al. 2024). Within tropical biotechnology, preclinical extract-level screening prioritizes safety margins, dose responsiveness, and organ-level biological effects prior to phytochemical standardization or compound isolation (Usman et al. 2023). Such investigations provide foundational data necessary for subsequent development pipelines, including quality control, formulation optimization, and mechanistic validation. Rather than serving as definitive therapeutic

evidence, these studies aim to identify promising biological candidates with reproducible effects under controlled experimental conditions, for which in vivo toxicological models such as AOM-induced organ injury offer practical and informative tools.

Momordica charantia (bitter melon) and *O. basilicum* (sweet basil) are widely used tropical medicinal plants with documented antioxidant, anti-inflammatory, and cytoprotective properties. *Momordica charantia* contains bioactive constituents such as flavonoids, phenolics, and cucurbitane-type triterpenoids associated with hepatoprotective and metabolic regulatory effects (Gayathry and John 2022; Singh et al. 2023). Similarly, *O. basilicum* is rich in polyphenols and essential oil components reported to modulate oxidative stress and inflammatory responses (Sarhan et al. 2019; Al-Snafi 2021). Previous experimental studies have demonstrated that extracts from these plants can attenuate chemically induced tissue injury, particularly in hepatic models; however, most investigations have focused on single-plant interventions, with limited attention to combined extract evaluation in systemic toxicity models.

Combining plant extracts with complementary biological properties represents a common strategy in phytoscience, aiming to enhance biological performance through additive or synergistic effects. In the case of *M. charantia* and *O. basilicum*, both plants exhibit antioxidant and anti-inflammatory activities that may collectively mitigate oxidative and inflammatory damage induced by AOM exposure. From a biotechnological perspective, evaluating combined extracts at the preclinical stage enables empirical assessment of overall biological trends and safety profiles prior to fractionation or compound-level investigation, without presupposing defined molecular mechanisms. The present study proposes the following hypothesis: Combined aqueous extracts of *M. charantia* and *O. basilicum* attenuate azoxymethane-induced systemic organ toxicity in rats, as evidenced by improved biochemical markers and reduced histopathological damage.

The present study was designed as a preclinical investigation to evaluate the biological potential of a combined aqueous extract of *M. charantia* and *O. basilicum* in an AOM-induced rat model. Specifically, the objectives were to (i) assess the acute safety profile of the combined extract, (ii) evaluate its effects on biochemical markers of liver function and histopathological features of extra-colonic organs, including the liver, kidney, and heart, and (iii) examine dose-dependent response patterns as foundational data for further biotechnological and phytochemical development. Importantly, this study is positioned not as a therapeutic efficacy assessment but as a preclinical multi-organ screening of a combined tropical plant extract under a chemically induced systemic toxicity model.

MATERIALS AND METHODS

Chemicals and reagents

Azoxymethane (AOM) was obtained from Sigma-Aldrich (St.Louis, MO, USA). Diagnostic assay kits for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were purchased from Randox Laboratories (UK). All other chemicals and reagents used in the study were of analytical grade and sourced from certified commercial suppliers.

Plant material collection, authentication, and extraction

Fresh leaves of *M. charantia* and *O. basilicum* were collected from open grassland areas in Sagamu, Ogun State, Nigeria. The plant materials were authenticated by a taxonomist in the Department of Plant Science, Olabisi Onabanjo University, Ago-Iwoye, Nigeria. Voucher specimens were deposited at the departmental herbarium for future reference. Only healthy, disease-free leaves were selected to ensure uniformity and reproducibility of the extraction process.

Collected leaves of *M. charantia* and *O. basilicum* were thoroughly washed under running tap water to remove debris and contaminants, followed by air-drying at room temperature until a constant weight was achieved. The dried plant materials were pulverized separately into fine powders using a mechanical grinder. Twenty grams of each powdered sample were extracted in 100 mL of distilled water at room temperature for 48 h with intermittent stirring. The mixtures were filtered using Whatman No.1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 45°C to obtain semi-solid residues. Extracts were stored at 4°C until use. The percentage yield was calculated based on the weight of dried extract relative to the initial plant material. The aqueous extraction approach was adopted as a conventional and reproducible method suitable for preclinical extract-level screening (Usman et al. 2023).

Experimental animals, housing, and ethical approval

Thirty-five healthy adult Wistar rats weighing 180-200 g were obtained from the Animal House of the Department of Physiology, Olabisi Onabanjo University, Remo Campus, Nigeria. Animals were housed in wire-mesh cages bedded with clean wood shavings under standard laboratory conditions (24-26°C, 12 h light/dark cycle). Rats were provided with commercial pelletized feed and water ad libitum and acclimatized for two weeks prior to the commencement of the experiment.

All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. The study adhered to institutional ethical standards of Olabisi Onabanjo University and followed principles aimed at minimizing animal suffering, reducing the number of animals used, and ensuring humane handling throughout the experimental period.

Acute toxicity assessment (LD_{50})

Acute toxicity of the combined aqueous extract of *M. charantia* and *O. basilicum* was evaluated using Lorke's method in two stages. In the first stage, nine rats were randomly divided into three groups (n = 3) and administered intraperitoneally with 10, 100, or 1,000 mg/kg of the combined extract (1:1, v/v). Animals were observed

for 24 h for signs of toxicity or mortality. In the second stage, three rats received higher doses of 1,500, 3,000, and 5,000 mg/kg, respectively, and were monitored for mortality. The intraperitoneal route was employed exclusively as a rapid and sensitive approach for preliminary safety screening to detect acute systemic toxicity, rather than to infer oral toxicity thresholds. Accordingly, the LD_{50} outcomes obtained via intraperitoneal administration are interpreted solely as an initial safety margin and are not extrapolated to the oral route used in the main experimental protocol, which was selected to reflect physiologically relevant exposure conditions.

Azoxymethane induction and experimental design

Azoxymethane was administered orally to induce systemic organ toxicity. Rats in the induced groups received AOM at a dose of 20 mg/kg body weight once weekly for four consecutive weeks. This dosing regimen was selected based on previous experimental studies demonstrating reproducible systemic and extra-colonic toxic effects following AOM exposure. Animals in the negative control group received standard feed and water without AOM administration throughout the experimental period.

Animals were randomly assigned to five experimental groups (n = 7 per group). Group 1 served as the negative control and received feed and water only. Group 2 served as the positive control and received AOM (20 mg/kg). Groups 3-5 constituted the treatment groups and received AOM (20 mg/kg) in combination with daily oral administration of the combined aqueous extract of *M. charantia* and *O. basilicum* (1:1, v/v) at doses of 200, 100, and 50 mg/kg body weight, respectively. Extract administration was carried out for 28 consecutive days using an oral cannula. The experimental design was intended to evaluate dose-dependent biological responses following combined extract exposure.

Sample collection, biochemical analysis, and histopathology

Sample collection and tissue processing

At the end of the experimental period, animals were euthanized using diethyl ether. Blood samples were collected via cardiac puncture for biochemical analysis. The liver, kidney, and heart were excised, rinsed in normal saline, and fixed in 10% buffered formalin. Fixed tissues were processed using standard paraffin-embedding procedures, sectioned at 5 µm thickness, and stained with hematoxylin and eosin for histopathological examination.

Biochemical analysis of liver function markers

Plasma samples obtained from centrifuged blood were used for biochemical analysis. Serum levels of AST, ALT, and ALP were determined using commercially available Randox diagnostic kits (Randox Laboratories, UK) according to the manufacturer's instructions. These parameters were selected as standard indicators of hepatocellular integrity and liver functional status.

Histopathological examination

Formalin-fixed liver, kidney, and heart tissues were processed using routine paraffin-embedding techniques. Tissue sections of 5 µm thickness were prepared using a microtome and mounted on glass slides. Sections were stained with hematoxylin and eosin and examined under a light microscope. Histological evaluation was descriptive and focused on structural integrity, cellular organization, vascular changes, and evidence of tissue degeneration or recovery.

Statistical analysis

All data were expressed as mean ± Standard Deviation (SD) for seven animals per group (n = 7). Statistical analysis was performed using one-way Analysis of Variance followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$. Analyses were conducted using GraphPad Prism version 9.0.

RESULTS AND DISCUSSION

Acute toxicity profile of the combined extract

The acute toxicity assessment of the combined aqueous extract of *M. charantia* and *O. basilicum* was conducted to evaluate its safety profile prior to the main experimental protocol. No mortality was observed in animals administered with doses ranging from 10 to 1,000 mg/kg during the first stage of the toxicity test. Similarly, no deaths were recorded in rats exposed to higher doses of 1,500, 3,000, and 5,000 mg/kg during the second stage of the assessment (Table 1). Animals across all dose levels remained active and did not exhibit observable signs of acute toxicity throughout the monitoring period. The absence of lethality at doses up to 5,000 mg/kg indicates a wide safety margin for the combined extract under the conditions of this study.

Effects on liver function biomarkers in AOM-induced rats

The effects of the combined aqueous extract of *M. charantia* and *O. basilicum* on liver function biomarkers in azoxymethane-induced rats are presented in Table 2. Administration of AOM alone (Group 2) resulted in marked alterations in hepatic biochemical parameters compared with the negative control group. Serum levels of AST, ALT, and ALP were substantially elevated in the AOM-only group, accompanied by reduced total protein and albumin concentrations.

Table 1. Acute toxicity profile of the combined aqueous extract of *Momordica charantia* and *Ocimum basilicum* following intraperitoneal administration (LD_{50} assessment)

Stage	Dose (mg/kg)	Number of deaths
One	10	0/3
	100	0/3
	1,000	0/3
Two	1,500	0/1
	3,000	0/1
	5,000	0/1

In contrast, rats treated with the combined extract alongside AOM (Groups 3-5) showed varying degrees of modulation of these biochemical markers. The group receiving 200 mg/kg extract exhibited lower AST, ALT, and ALP values relative to the AOM-only group, with corresponding increases in total protein and albumin levels. Similar trends were observed in the 100 mg/kg and 50 mg/kg treatment groups, although enzyme levels remained higher than those of the negative control group. Overall, the biochemical data demonstrate dose-related variations in liver function markers following extract administration in AOM-induced rats (Table 2).

Histopathological features of hepatic tissue

Histopathological examination of liver sections revealed distinct structural differences among the experimental groups (Figure 1). Liver tissue from the negative control group showed preserved hepatic architecture characterized by well-arranged hepatocyte plates, clearly defined central veins, and intact sinusoidal spaces. In contrast, liver sections from the AOM-only group exhibited pronounced structural alterations, including hepatocellular distortion, vascular congestion, disrupted sinusoidal organization, and evidence of cellular degeneration.

Rats treated with the combined extract demonstrated variable histological patterns depending on the administered dose. Liver sections from animals receiving 200 mg/kg extract showed comparatively preserved tissue organization with reduced structural disruption. Sections from the 100 mg/kg group displayed moderate architectural irregularities, whereas those from the 50 mg/kg group exhibited persistent histological alterations similar to, but less extensive than, the AOM-only group. These observations indicate dose-related variations in hepatic tissue morphology following extract administration in AOM-induced rats (Figure 1).

Table 2. Effects of combined aqueous extracts of *Momordica charantia* and *Ocimum basilicum* on liver function biomarkers in azoxymethane-induced rats (mean ± SD, n = 7)

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Total protein (g/dL)	Albumin (g/dL)
Group 1 (Negative control)	45 ± 6^{a}	38 ± 5^{a}	110 ± 9^{a}	2.4 ± 0.2^{a}	1.4 ± 0.3^{a}
Group 2 (AOM only)	120 ± 10^{b}	95 ± 8^{b}	220 ± 15^{b}	1.6 ± 0.3^{b}	0.9 ± 0.2^{b}
Group 3 (200 mg/kg + AOM)	68 ± 8^{a}	52 ± 6^{a}	130 ± 11^{a}	3.2 ± 0.3^{b}	2.1 ± 0.3^{a}
Group 4 (100 mg/kg + AOM)	84 ± 7^{b}	59 ± 7^{b}	165 ± 12^{b}	3.5 ± 0.4^{b}	2.0 ± 0.2^{a}
Group 5 (50 mg/kg + AOM)	90 ± 9^{b}	63 ± 8^{b}	210 ± 14^{b}	3.8 ± 0.5^{b}	2.2 ± 0.3^{a}

Note: Values represent mean ± SD. Different superscript letters within the same column indicate significant differences at $p < 0.05$

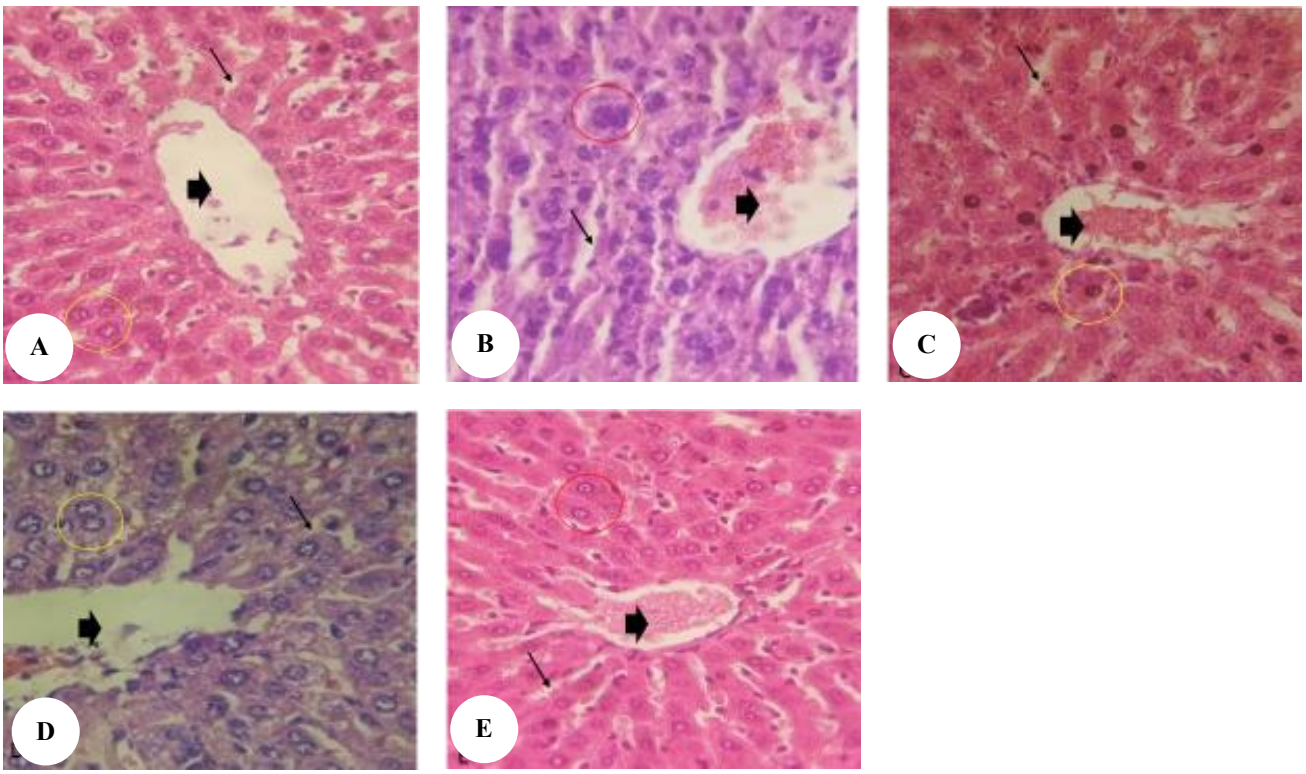

Figure 1. Representative histological features of hepatic tissue following treatment with combined aqueous extracts of *Momordica charantia* and *Ocimum basilicum* in azoxymethane-induced rats (Hematoxylin and eosin staining, ×400). A. Negative control, B. AOM-induced, C. Extract 200 mg/kg + AOM, D. Extract 100 mg/kg + AOM, E. Extract 50 mg/kg + AOM. Yellow circle: Hepatic plates, Black thin arrow: Sinusoids with kupffer cells, Black thick arrow: Central vein, Red circle: Hepatocyte, Arrows: Nuclei, Circles: Junctions

Histopathological features of cardiac tissue

Histological evaluation of cardiac tissue revealed marked structural differences among the experimental groups (Figure 2). Sections obtained from the negative control group exhibited normal myocardial architecture, characterized by well-organized cardiac muscle fibers, intact nuclei, and clearly visible intercalated discs. In contrast, cardiac sections from the AOM-only group demonstrated noticeable histopathological alterations, including disorganized muscle fibers, nuclear distortion, and inflammatory cell infiltration.

Treatment with the combined aqueous extract resulted in variable cardiac tissue morphology depending on the administered dose. Sections from rats receiving 200 mg/kg extract showed relatively preserved myocardial structure with reduced fiber disorganization. Moderate structural irregularities were observed in the 100 mg/kg treatment group, whereas the 50 mg/kg group exhibited persistent myocardial alterations comparable to those observed in the AOM-only group. These findings indicate dose-related variations in cardiac tissue architecture following extract administration in AOM-induced rats (Figure 2).

Histopathological features of renal tissue

Histopathological examination of renal tissue revealed distinct morphological differences across the experimental groups (Figure 3). Kidney sections from the negative control group displayed normal renal architecture, including well-defined glomeruli, intact capsular spaces, and clearly organized proximal and distal convoluted tubules. In contrast, the AOM-only group exhibited pronounced renal alterations, characterized by glomerular degeneration, reduced capsular space, and tubular distortion.

Rats treated with the combined extract showed dose-related variations in renal tissue morphology. Sections from the 200 mg/kg group demonstrated comparatively preserved renal structure with improved glomerular integrity and tubular organization. The 100 mg/kg group displayed moderate histological irregularities, whereas the 50 mg/kg group showed persistent renal alterations similar to those observed in the AOM-only group. These observations reflect dose-dependent differences in renal tissue morphology following extract administration in AOM-induced rats (Figure 3).

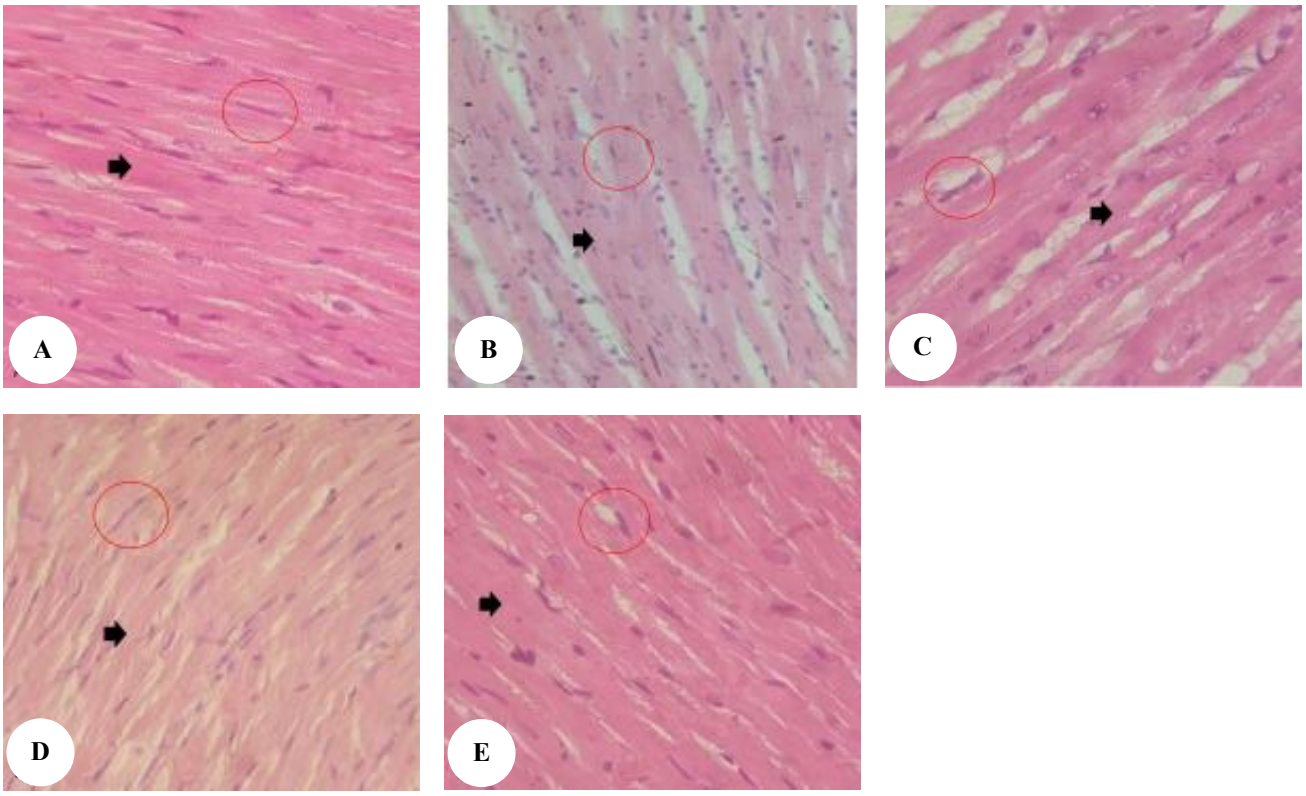

Figure 2. Representative histological features of cardiac tissue following treatment with combined aqueous extracts of *Momordica charantia* and *Ocimum basilicum* in azoxymethane-induced rats (Hematoxylin and eosin staining, ×400). A. Negative control, B. AOM-induced, C. Extract 200 mg/kg + AOM, D. Extract 100 mg/kg + AOM, E. Extract 50 mg/kg + AOM. Arrows: Nuclei, Circles: Intercalated junctions

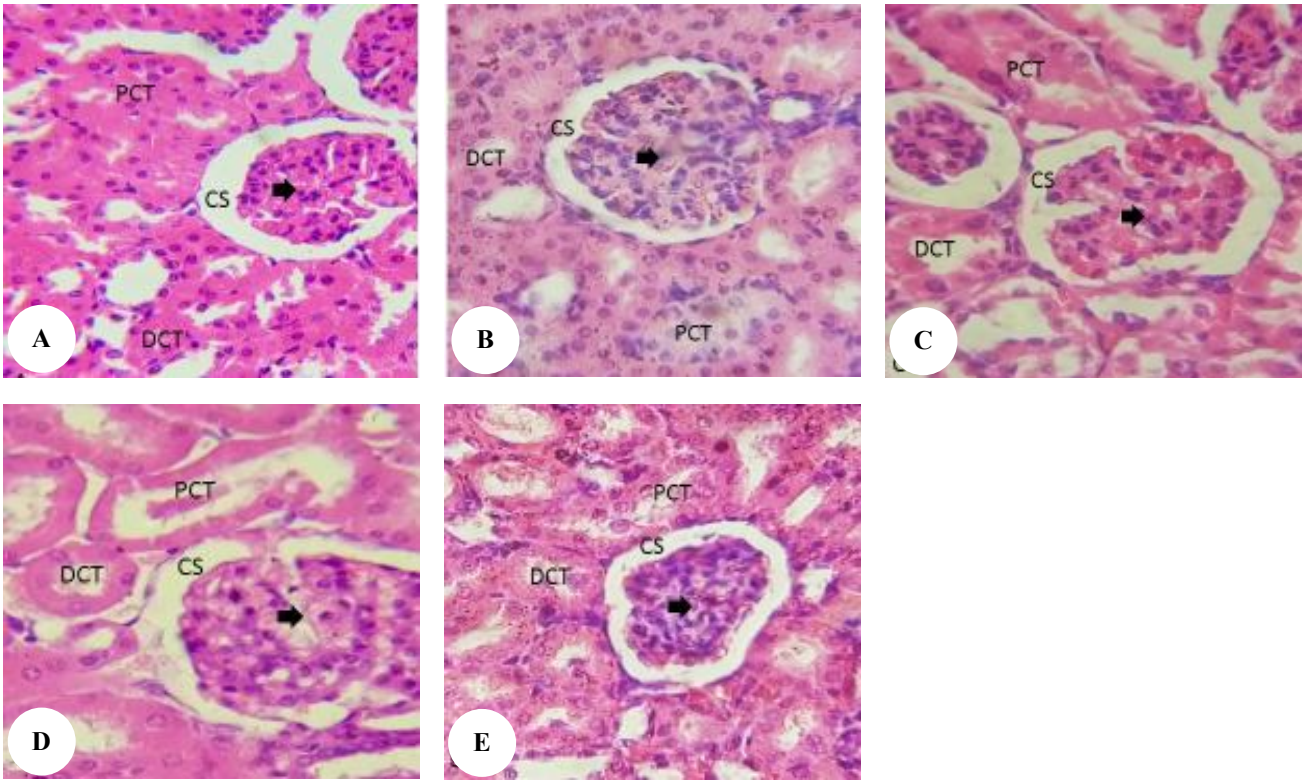

Figure 3. Representative histological features of renal tissue following treatment with combined aqueous extracts of *Momordica charantia* and *Ocimum basilicum* in azoxymethane-induced rats (Hematoxylin and eosin staining, ×400). A. Negative control, B. AOM-induced, C. Extract 200 mg/kg + AOM, D. Extract 100 mg/kg + AOM, E. Extract 50 mg/kg + AOM. CS: Capsular Space, PCT: Proximal Convulated Tubules, DCT: Distal Convulated Tubules

Discussion

Safety profile and implications for preclinical extract screening

Evaluation of safety is a critical prerequisite in preclinical screening of plant-derived extracts, particularly within the context of tropical biotechnology, where extract-level formulations often precede compound isolation. In the present study, the combined aqueous extract of *M. charantia* and *O. basilicum* exhibited no observable mortality at doses up to 5000 mg/kg in acute toxicity testing. This outcome is consistent with previous reports describing low acute toxicity profiles for *M. charantia* and *O. basilicum* when administered individually in rodent models (Al-Snafi 2021; Gayathry and John 2022). Similar findings have been reported for other tropical medicinal plants evaluated using Lorke's method, where the absence of lethality at high doses indicates a wide safety margin suitable for further biological assessment (Moudal et al. 2023).

From a toxicological classification perspective, extracts with LD_{50} values exceeding 5000 mg/kg are generally regarded as practically non-toxic under acute exposure conditions (OECD 423 2001; OECD 425 2008). Although acute toxicity does not predict chronic or target-organ toxicity, such findings provide foundational evidence supporting the suitability of the extract for subsequent subacute and organ-specific evaluations. In biotechnological screening pipelines, this stage functions as an exclusion filter, eliminating candidates with narrow safety margins before resource-intensive analyses such as phytochemical profiling or mechanistic assays (Atanasov et al. 2021; Mugale et al. 2024).

Importantly, the absence of acute toxicity in the combined extract suggests that extract-extract interaction did not introduce immediate adverse effects, supporting its feasibility as a candidate formulation for early-stage biological screening rather than definitive therapeutic application.

Organ-specific protective trends following extract administration

The present findings demonstrate distinct organ-specific trends in response to administration of the combined aqueous extract of *M. charantia* and *O. basilicum* in azoxymethane-induced rats.AOM exposure resulted in pronounced hepatic, renal, and cardiac alterations, consistent with its known systemic toxicity profile mediated by reactive metabolites and oxidative stress (Megaraj et al. 2014; Waggie et al. 2022). Elevation of liver enzymes and disruption of tissue architecture observed in the AOM-only group align with previous reports describing hepatocellular membrane damage and impaired metabolic function following AOM administration (Matkowskyj et al.1999; Lima et al. 2019).

Upon extract administration, organ-specific variations were evident, with hepatic tissue showing the most consistent modulation across biochemical and histological

parameters. Reduction of AST, ALT, and ALP levels, accompanied by improved hepatic architecture, suggests attenuation of hepatocellular leakage and partial preservation of liver integrity. Similar hepatoprotective trends have been reported for *M. charantia* and *O. basilicum* extracts in chemically induced liver injury models, where antioxidant and cytoprotective properties were associated with stabilization of cellular membranes (Sarhan et al. 2019; Thilagavathi et al. 2023). Comparable outcomes have also been described for other tropical plant extracts evaluated in preclinical toxicological models (Ajiboye et al. 2014).

An additional observation is that serum albumin and total protein levels in extract-treated groups exceeded those of the negative control. This finding should not be interpreted as evidence of a direct anabolic or protein synthesis-stimulating effect of the extract. Rather, it likely reflects indirect restoration or rebound of hepatic synthetic capacity following AOM-induced suppression of liver function. Azoxymethane exposure is known to impair hepatocellular metabolism and protein synthesis through oxidative and metabolic stress. Mitigation of ongoing hepatic injury may therefore permit normalization or transient elevation of circulating protein levels relative to untreated controls under short-term experimental conditions. In the absence of molecular or metabolic analyses directly assessing anabolic pathways, these changes are conservatively interpreted as indicators of improved hepatic functional status rather than enhanced de novo protein synthesis, consistent with the extract-level screening nature of the present study.

Renal tissue responses followed a comparable but less pronounced pattern. Structural preservation of glomeruli and tubular organization in extract-treated groups contrasts with the extensive degeneration observed in AOM-only rats, reflecting partial mitigation of nephrotoxic effects. This observation is consistent with studies linking plant-derived antioxidants to reduced renal oxidative stress and tubular damage in toxin-induced nephropathy models (Erseckin et al. 2022). Cardiac tissue exhibited more modest structural modulation, which may reflect indirect protection via systemic redox balance rather than direct cardiotropic effects, as previously suggested in experimental models of chemically induced cardiotoxicity (Sonowal et al. 2017; Cadeddu et al. 2018).

Collectively, these organ-specific trends indicate that the combined extract exerts differential biological effects across tissues, with the liver showing the highest responsiveness, followed by the kidney and heart. Such patterns are typical in systemic toxicological models and support the use of multi-organ assessment in preclinical extract screening.

Dose-response patterns and biological consistency

Dose-dependent trends observed in the present study provide an important layer of biological consistency supporting the reliability of the experimental findings. Administration of the combined aqueous extract at increasing doses was associated with progressively moderated biochemical and histopathological alterations in AOM-induced rats, particularly within hepatic tissue. Such graded responses are commonly interpreted as indicators of biological plausibility in preclinical toxicological screening, rather than definitive evidence of mechanistic action (Calabrese and Mattson 2017; Atanasov et al. 2021).

In the liver, the highest extract dose demonstrated the most pronounced modulation of enzyme leakage markers and structural preservation, whereas lower doses showed partial but consistent trends in the same direction. Similar dose-related patterns have been reported in studies evaluating *M. charantia* extracts in chemically induced liver injury, where higher concentrations were required to achieve measurable stabilization of hepatocellular integrity (Gayathry and John 2022; Kunnaja et al. 2026). Comparable observations have also been documented for *O. basilicum* and other polyphenol-rich tropical plants, supporting the concept that extract-level efficacy often depends on sufficient bioactive availability (Al-Snafi 2021; Imtiaz et al. 2025).

Across renal and cardiac tissues, dose-response relationships were less sharply defined but remained directionally consistent, suggesting tissue-specific sensitivity and differential exposure to reactive metabolites. Such variability aligns with systemic toxicological models, where organ responsiveness is influenced by metabolic capacity, vascularization, and intrinsic antioxidant defenses (Megaraj et al. 2014). Overall, the presence of internally consistent dose-related trends strengthens the interpretation of the extract's biological relevance while remaining within the limits of descriptive preclinical evidence.

Interpretation of histopathological recovery patterns

Histopathological evaluation provides a structural context to the biochemical alterations observed in systemic toxicological models. In the present study, liver, kidney, and heart tissues from AOM-induced rats exhibited architectural disruptions consistent with chemically induced organ injury, including cellular distortion, vascular congestion, and loss of normal tissue organization. These features are well documented in AOM-based models and are generally attributed to oxidative stress, inflammatory responses, and metabolite-mediated cytotoxicity (Matkowskyj et al. 1999; Waggie et al. 2022).

Following administration of the combined extract, histological patterns indicated varying degrees of structural preservation across organs. Importantly, these changes should be interpreted as attenuation of tissue damage rather than true regeneration. Preservation of hepatocyte arrangement, reduced congestion, and improved tubular organization in extract-treated groups likely reflect mitigation of ongoing cellular injury, a phenomenon commonly reported in plant-based toxicological studies (Hassan et al. 2017; Thilagavathi et al. 2023). Similar descriptive improvements have been observed in renal and cardiac tissues exposed to oxidative or chemical insults when antioxidant-rich plant extracts are administered (Sonowal et al. 2017; Erseckin et al. 2022).

It is also important to note that histopathological assessment in this study was qualitative and descriptive, without morphometric quantification. As such, observed

structural differences should be viewed as supportive evidence complementing biochemical findings, rather than standalone proof of tissue repair. This interpretive restraint aligns with best practices in preclinical toxicology, where histology serves to contextualize biochemical trends rather than establish mechanistic conclusions (OECD 407 2008; Suvarna et al. 2018).

Relevance to tropical biotechnology and phytoscience

Within the context of tropical biotechnology, the present study contributes to early-stage biological screening of plant-derived extracts rather than definitive therapeutic validation. Tropical regions harbor extensive plant biodiversity, yet only a limited proportion of medicinal species have undergone systematic safety and organ-level evaluation under controlled experimental conditions (Cordell and Colvard 2012; Atanasov et al. 2021). In this regard, extract-level studies that integrate acute safety assessment, biochemical profiling, and multi-organ histopathology provide essential baseline data for downstream biotechnological development.

The combined evaluation of *M. charantia* and *O. basilicum* aligns with current phytoscience approaches that prioritize functional screening prior to phytochemical standardization or bioactive isolation. Demonstration of a wide safety margin, dose-related biological consistency, and reproducible organ-level trends supports the feasibility of this extract combination as a candidate for further investigation. Importantly, such findings do not imply clinical applicability but instead inform decisions related to extract optimization, quality control, and prioritization for mechanistic studies.

From a translational perspective, the study underscores the value of multi-organ assessment in preclinical screening frameworks, particularly for plant extracts intended for long-term or systemic use. By situating the findings within a biotechnological screening pipeline, this work contributes to the rational utilization of tropical medicinal plants as biological resources rather than unvalidated therapeutic agents (Fabricant and Farnsworth 2001; Mugale et al. 2024).

Limitations of the study

Several limitations of the present study should be acknowledged when interpreting the findings. First, the use of aqueous extraction may have restricted the recovery of certain bioactive constituents with low water solubility, potentially underrepresenting the full phytochemical spectrum of *M. charantia* and *O. basilicum*. Second, no phytochemical profiling or quantitative standardization of the extract was performed; therefore, specific compounds responsible for the observed biological trends could not be identified. Third, mechanistic assays related to oxidative stress, inflammatory pathways, or molecular signaling were not included, limiting interpretation to organ-level and biochemical observations. In addition, histopathological assessment was qualitative and descriptive, without morphometric or scoring-based quantification. Finally, the study was limited to acute and short-term exposure and does not provide insight into long-term safety or chronic effects. These constraints indicate that the findings should be viewed as preliminary and supportive of further biotechnological investigation rather than conclusive evidence of therapeutic efficacy.

In conclusion, this study provides preclinical evidence supporting the biological relevance of a combined aqueous extract of *M. charantia* and *O. basilicum* in an azoxymethane-induced model of systemic organ toxicity. Acute toxicity assessment showed that the combined extracts were safe, with an $LD_{50} > 5000$ mg/kg, indicating a wide safety margin. AOM exposure produced marked biochemical disturbances, including significant elevations in ALT, AST, ALP, urea, and creatinine, together with histopathological damage in the liver, kidney, and heart. These findings suggest that the combined extract exhibits organ-specific protective trends, with the liver showing the highest responsiveness under the experimental conditions employed. Importantly, the results should be interpreted within the context of extract-level biological screening rather than definitive therapeutic validation. From a tropical biotechnology perspective, the integration of safety profiling, biochemical assessment, and multi-organ histopathology provides foundational data to inform further phytochemical characterization, extract standardization, and mechanistic investigation. Overall, the study supports the potential of this plant combination as a candidate for subsequent stages of biotechnological and phytoscientific development. Future studies should incorporate detailed phytochemical characterization, mechanistic investigations (e.g., oxidative stress and inflammatory markers), longer-term exposure models, and dose-optimization experiments. Such work will be essential to validate efficacy, clarify mechanisms of action, and support further development of these tropical plant extracts within a preclinical biotechnology framework.

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