Modulatory Effects of Moringa Oleifera and Musa Sapentium on P53 Expression Changes in Dmba And Cadmium-Induced Hepatotoxicity in Male Wistar Rats

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ABSTRACT

Chemical-induced hepatotoxicity remains a major concern due to environmental and experimental exposure to toxicants such as 7,12-dimethylbenz[a]anthracene (DMBA) and cadmium. Medicinal plants with antioxidant properties may offer protective benefits against liver injury. This study investigated the ameliorative effects of Moringa oleifera and Musa sapentium on DMBA and cadmium-induced hepatotoxicity in male Wistar rats, with emphasis on hepatic p53 modulation and histopathological changes. Male Wistar rats were exposed to DMBA and cadmium, either alone or in combination with Moringa oleifera or Musa sapentium extracts. Hepatic p53 levels were quantified using ELISA, while liver tissues were examined histologically using hematoxylin and eosin staining. Administration of DMBA and cadmium resulted in significant alterations in hepatic p53 levels and severe histopathological damage to liver tissue. Co-treatment with Moringa oleifera or Musa sapentium extracts modulated toxicant-induced p53 changes and improved liver histoarchitecture compared with toxicant-only treated groups. The findings suggest that Moringa oleifera and Musa sapentium exert protective effects against DMBA- and cadmium-induced hepatotoxicity, potentially through modulation of p53-associated stress responses and preservation of liver tissue integrity.

Keywords: p53, Moringa oleifera, Musa sapentium, DMBA, Cadmium.

INTRODUCTION AND LITERATURE REVIEW

The liver plays a central role in the metabolism and detoxification of xenobiotics, making it particularly vulnerable to injury following exposure to environmental and experimental toxicants. Chemical-induced hepatotoxicity remains a major concern in toxicological research due to its association with oxidative stress, cellular degeneration, and disruption of critical metabolic and regulatory pathways (Martínez-Sena et al., 2023). Prolonged or repeated exposure to hepatotoxic agents can result in structural and functional alterations of hepatic tissue, ultimately predisposing cells to chronic liver disease and carcinogenic transformation (Wang et al., 2025).

7,12-Dimethylbenz(a)anthracene (DMBA) is a well-established polycyclic aromatic hydrocarbon widely employed in experimental models to induce hepatic toxicity and carcinogenesis (Adefisan-Adeoye et al., 2025). Its metabolic activation generates reactive intermediates capable of inducing oxidative stress, DNA damage, and abnormal cellular proliferation (Adefisan-Adeoye et al., 2025). Similarly, cadmium is a pervasive environmental heavy metal with a high affinity for hepatic accumulation. Cadmium exposure has been shown to disrupt redox balance, impair antioxidant defense systems, and promote hepatocellular injury through lipid peroxidation and inflammatory processes (Farahat et al., 2025). The liver therefore represents a primary target organ for both DMBA- and cadmium-induced toxicity.

Despite extensive use of these toxicants in experimental models, effective strategies for mitigating their hepatotoxic effects remain limited. Current therapeutic interventions are often constrained by adverse effects or inadequate efficacy, highlighting the need for alternative approaches that can attenuate toxicant-induced liver damage while preserving normal cellular function (Chen et al., 2025). This has driven increasing interest in biologically active natural products as potential protective agents against chemically induced hepatotoxicity.

Molecular Mechanisms and the Role of p53

At the molecular level, toxicant-induced hepatotoxicity is closely associated with oxidative stress–mediated DNA damage and disruption of cellular regulatory pathways (Wang et al., 2025). Reactive oxygen species

generated during the metabolism of chemical toxicants such as DMBA and cadmium can compromise genomic integrity, alter signal transduction cascades, and trigger maladaptive cellular responses (Badawi et al., 2024). When these insults overwhelm endogenous defense mechanisms, they may lead to aberrant cell cycle progression, impaired apoptosis, and enhanced susceptibility to malignant transformation.

The tumour suppressor protein p53 plays a pivotal role in maintaining cellular homeostasis under conditions of toxic and genotoxic stress. Acting as a critical regulator of the cell cycle, p53 coordinates DNA repair, cell cycle arrest, and apoptosis in response to cellular damage (Zhao et al., 2024). In the liver, p53 serves as an important molecular sentinel, integrating stress signals to prevent the propagation of damaged hepatocytes (Zhao et al., 2024). Alterations in p53 expression or activity have therefore been widely used as indicators of toxicant-induced cellular dysfunction and carcinogenic risk in experimental models (Wang et al., 2023).

Adegbola & Adetutu (2024) toxicological studies indicates that exposure to polycyclic aromatic hydrocarbons and heavy metals can significantly modulate p53 signaling pathways. DMBA has been shown to induce DNA adduct formation and genomic instability, events that place substantial regulatory demands on p53-mediated responses (Adeoye et al., 2023). Cadmium, although not directly genotoxic, indirectly interferes with DNA repair systems and redox balance, leading to altered p53 regulation and impaired cellular defense (Qu & Zheng, 2024). Dysregulation of p53 under such conditions may compromise the liver's ability to effectively respond to toxic injury, thereby exacerbating tissue damage and disease progression.

Given its central role in coordinating cellular responses to chemical stress, p53 represents a sensitive and biologically relevant biomarker for assessing both toxicant-induced liver injury and the efficacy of protective interventions (Wittke, 2025). Evaluation of p53 modulation alongside histopathological changes therefore provides a mechanistic framework for understanding hepatotoxicity and potential ameliorative effects in experimental models.

Medicinal Plants as Protective Agents

Medicinal plants have long been recognized as important sources of therapeutic agents for the prevention and treatment of disease. Traditional medicine, which predates modern pharmacology, derives its foundation from accumulated indigenous knowledge and empirical use of plants for curative, protective, and promotive purposes (Aremu et al., 2024). Even in contemporary healthcare systems, plant-derived products remain central to drug discovery and disease management, particularly in regions where access to synthetic medicines is limited.

Fatima et al. (2025) found out that despite the availability of synthetic drugs, their long-term use is often associated with adverse effects, high cost, and limited efficacy against chronic diseases, including those involving oxidative stress and chemical-induced organ damage. Consequently, there has been renewed scientific interest in medicinal plants as alternative or complementary therapeutic agents. A whole of plant extracts are believed to exert biological effects through synergistic interactions among their phytochemical constituents, a property that cannot always be replicated by isolated compounds (Yadav et al., 2024). This holistic activity has contributed to the sustained relevance of medicinal plants in toxicological and pharmacological research.

Etukudo et al. (2025) also found out that Plant-derived phytochemicals such as flavonoids, phenolics, alkaloids, tannins, terpenoids, and glycosides have been shown to possess antioxidant, anti-inflammatory, antimicrobial, and chemopreventive properties. These compounds play a crucial role in scavenging reactive oxygen species, stabilizing cellular membranes, modulating enzyme activity, and protecting tissues against oxidative and chemical injury. In the context of hepatotoxicity, such properties are particularly relevant, as oxidative stress is a central mechanism underlying liver damage induced by environmental and experimental toxicants.

Mugale et al. (2024) also found out that although medicinal plants are often perceived as inherently safe, their bioactive constituents can exert both therapeutic and toxic effects depending on dosage, duration of exposure, and method of preparation. This underscores the importance of toxicological evaluation of medicinal plant extracts, especially when proposed for long-term or therapeutic use. Experimental studies assessing biochemical, histopathological, and molecular parameters therefore remain essential for validating the safety and protective efficacy of plant-based interventions.

Given these considerations, medicinal plants represent promising candidates for mitigating chemically induced hepatotoxicity, particularly through antioxidant and cytoprotective mechanisms. Their ability to modulate

oxidative stress pathways and cellular defense systems provides a scientific basis for investigating their potential protective roles against liver injury induced by toxic agents.

Rationale for the Selection of Moringa oleifera and Musa sapentium

Moringa oleifera and Musa sapentium were selected for this study based on their extensive traditional use, rich phytochemical composition, and reported biological activities relevant to the prevention of chemically induced liver injury. These plants are widely distributed, readily available, and commonly utilized in traditional medicine for nutritional supplementation and therapeutic purposes, particularly in the management of metabolic disorders and diseases associated with oxidative stress.

Moringa oleifera is recognized for its high nutritional value and diverse medicinal properties. Various parts of the plant, especially the leaves, have been reported to contain abundant bioactive compounds including flavonoids, phenolics, vitamins, carotenoids, and other antioxidant constituents (Su et al., 2023). These phytochemicals contribute to its documented antioxidant, anti-inflammatory, and hepatoprotective properties. Within the context of toxicological exposure, the antioxidant capacity of *Moringa oleifera* suggests a potential role in mitigating oxidative stress-mediated liver damage and preserving hepatic cellular integrity following exposure to chemical toxicants.

Musa sapentium (banana) has also been traditionally employed for medicinal and nutritional purposes. The leaves and other parts of the plant are known to contain biologically active compounds such as flavonoids, triterpenes, and phenolic substances, which have been associated with antioxidant and cytoprotective activities (Gervásio & Batitucci, 2023). These properties indicate that *Musa sapentium* may contribute to the stabilization of cellular membranes, reduction of lipid peroxidation, and enhancement of endogenous defense mechanisms in tissues exposed to toxic insults.

Although both plants have been widely studied for their general antioxidant and therapeutic benefits, there remains limited experimental evidence regarding their effects on molecular biomarkers associated with toxicant-induced liver injury, particularly tumour suppressor pathways. To be precise, data on the modulation of p53, a key regulator of cellular stress responses, apoptosis, and DNA repair, in the context of DMBA and cadmium-induced hepatotoxicity are scarce. This represents an important gap in understanding the mechanistic basis of the hepatoprotective effects of these medicinal plants.

This study therefore investigated the ameliorative effects of Moringa oleifera and Musa sapentium on DMBA and cadmium-induced hepatotoxicity in male Wistar rats, with emphasis on p53 modulation and liver histopathology.

MATERIALS AND METHODS

Preparation of Plant Sample

The extracts of *Moringa oleifera, Musa sapentium* were used for this study. The powdered plant materials were subjected to extraction using the soxlet extractor. The powdered samples were soaked in the appropriate solvent and agitated intermittently to enhance extraction. The extracts were filtered and concentrated using standard procedures to obtain the crude extracts. The concentrated extracts were stored under suitable conditions until required for administration.

Chromatography

Column chromatography (CC) was carried out on silica gel (70-230 and 240-300 mesh size, Merck, Germany), Merck alumina (70 – 230 mesh ASTM). Thin Layer Chromatography (TLC) was carried out on pre – coated silica gel 60 F_{254} aluminum foil (Merck, Germany) for the establishment of the purity of isolates. Spots on TLC were examined with a UV lamp operating at a wavelength of 366nm for fluorescence and at 254nm for fluorescence quenching spots.

Fractionation of the Crude Extract

The extract was fractionated in a silica gel open column, using n – hexane, dichloromethane, ethyl acetate and methanol in an increasing order of polarity to make thirty – five eluents of 250 ml each. Eluents with similar

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TLC profile were pooled to give a total of seven combined fractions.

Phytochemical Screening

Phytochemical analysis of the extracts was carried out to ascertain the various classes of organic compounds present in the extract, for qualitative detection of alkaloids, flavonoids, tannins and saponins, steroids, phenols and glycosides according to the standard procedures (Harbone, 1998, Trease and Evans, 2009 and Kokate, 1997).

Test for Alkaloids (Dragendorrf test)

Approximately fifty (50) mg of each of the fractions was dissolved in a sufficient amount of distilled water. Concentrated hydrochloric acid (HCl) was then added to each of the solutions and the mixture filtered. Two (2) ml of this filtrate was collected in a test tube and (1) ml of dragendorrf's reagent was added along the inner wall of the test vessels.

Test for Saponins (Foam test)

Five (5) ml of each of the fractions were taken in a test tube and vigorously agitated for a period of five (5) minutes.

Detection of Flavonoids (Alkaline reagent test)

Five (5) drops of five (5) % sodium hydroxide solution was added to one (1) ml of each of the fractions, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

Detection of Tannins

The test reagent ferric chloride was prepared by dissolving 1g of $FeCl_3$ in 100mls of distilled water. 2 drops of the prepared reagent was added to 1ml of sample. A blue-black color was taken as evidence for the presence of tannin.

Test for Glycosides

Twenty –five (25) ml of dilute sulphuric acid was added to five (5) ml of the each of the fractions in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5ml of Fehling solution A and B was added. A brick red precipitate of reducing sugar indicates the presence of glycosides.

Test for Steroids

The plant extracts (1 mg) was taken in a test tube and dissolved with 10 ml of chloroform, then equal volume of concentrated sulphuric acid was added to the test tube by sides. The upper layer in the test tube turned into red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for Terpenoids

The plant extract (5 mg) was dissolved in 2 ml of chloroform, and then 1ml of acetic anhydride was added to it. Concentrated sulphuric acid (1 ml) was added to the solution. Formation of reddish violet colour showed the presence of terpenoids.

Test for Phenol

Exactly (0.5g) of extract was added with 3 to 4 ml of $FeCl_3$. The formation ofbluish black color indicates the presence of phenol in the extract.

Preparation Of Solutions of Drugs and Extracts

Preparation of 0.25M Sucrose Solution

85.75g of sucrose was measured into a beaker and dissolved with small quantity of distilled water. The solution was stirred for proper dissolution and the resulting solution was transferred into a 1-liter standard volumetric

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flask and made up to mark with distilled water.

Preparation of Sodium arsenite Solution

10mg/kg bw of Sodium arsenite, diluted in distilled water was administered for each rat receiving sodium arsenite, calculated in regard to its daily weight.

Preparation of Lead acetate Solution

100mg/kg bw of lead acetate, diluted in distilled water was administered for each rat receiving sodium arsenite, calculated in regard to its daily weight.

Method of administration

Chemicals; DMBA (7,12-dimethylbenz(a)anthracene), Cadmium, Sodium arsenite, Lead acetate (Sigma-Aldrich CHEMIE GmbH), plant extract (*Moringa oleifera, Musa sapentium*) were dissolved in distilled water, to allow for oral administration. Administration of solutions (chemicals and extracts) to rats across all groups was done orally using oral cannula except cadmium which was administered intraperitoneally.

Animal grouping and treatment

Eighty-five male wistar rats (average weight of 200g) were used for the study. The rats were randomly assigned into; a control together with sixteen groups for males with each group containing 5 rats. The rats in the control groups were administered physiological saline. The sixteen treatment groups received either DMBA, cadmium, sodium arsenite, lead acetate, *Moringa oleifera, Musa sapentium,* or in co-administration (Table 2.1). Weight of each rat was taken using Gallenkramp weighing balance. The detailed summary of the treatments is shown in table 2.1.

Grouping

Eighty five male wistar rats were grouped into seventeen (each group having 5 rats) as shown in the table 2.1 below.

Table 2.1: Grouping and administration of drugs/extracts in male rats

Group	Doses of Drugs / Extracts Administered
1	1 mL/200 g body weight of normal saline only for 8 weeks
2	Single oral administration of 15 mg/kg body weight of DMBA in week 1; monitored for 8 weeks
3	Single intraperitoneal injection of 1.25 mg/kg body weight of cadmium
4	Co-administration of 30 mg/kg body weight of Moringa oleifera for 8 weeks and single oral administration of 15 mg/kg body weight of DMBA in week 1
5	Co-administration of 10 mg/kg body weight of Musa sapientum and 1.25 mg/kg body weight of cadmium
6	Single oral administration of 15 mg/kg body weight of DMBA in week 1, followed by treatment with 15 mg/kg body weight of Moringa oleifera for 8 weeks
7	Single oral administration of 15 mg/kg body weight of DMBA in week 1, followed by treatment with 30 mg/kg body weight of Moringa oleifera for 8 weeks
8	Single oral administration of 10 mg/kg body weight of DMBA in week 1, followed by treatment with 10 mg/kg body weight of Musa sapientum for 8 weeks
9	Co-administration of 1.25 mg/kg body weight of cadmium and 15 mg/kg body weight of Moringa oleifera
10	Co-administration of 1.25 mg/kg body weight of cadmium and 30 mg/kg body weight of Moringa oleifera
11	Co-administration of 1.25 mg/kg body weight of cadmium and 10 mg/kg body weight of Musa sapientum

12	15 mg/kg body weight of DMBA and standard doses of 5-fluorouracil, cyclophosphamide, adriamycin, and mitomycin
13	1.25 mg/kg body weight of cadmium and standard doses of 5-fluorouracil, cyclophosphamide, adriamycin, and mitomycin
14	15 mg/kg body weight of Moringa oleifera only for 8 weeks
15	30 mg/kg body weight of Moringa oleifera only for 8 weeks
16	10 mg/kg body weight of Musa sapientum only for 8 weeks
17	Co-administration of 1.25 mg/kg body weight of cadmium and 30 mg/kg body weight of Moringa oleifera

bw=body weight.

Positive control: Group 1.

Negative controls: Group 2 and 3.

Hepato-protective potentials of plants: Groups 4,5,17.

Therapeutic Anti-hepatotoxicity potentials of plants: Rats of Groups 6-13 shall receive oral administration of DMBA or cadmium, followed by treatment with extract.

Toxicity profiling of plants: Groups 14-16

Sacrifice and sample collection

Rats of control and experimental groups (1 - 17) of males were sacrificed, twenty-four (24) hours after the last administration by cervical dislocation (to eliminate the interference of ketamine induced change in biochemical redox).

Biological Activity

The biological assay in this study was carried out at the department of anatomy, University of Ilorin.

Isolation and homogenization of tissues

The livers used for this study were excised and divided. The first part was weighed and placed in 10% formalin for histopathological examination while the second part of the liver was isolated and then subjected to homogenization using mortar and pestle in ice-cold 0.25M sucrose, in the proportion of 1g to 4ml of 0.25M sucrose solution. The homogenates were filled up to 5ml with additional sucrose and were stored in sample tubes and kept in the freezer until required for further analyses. Rinsed in 0.25 M sucrose 3 times for 5 minutes each and placed in 30% sucrose in which they were stored at 4°C, until required for further analyses.

Histopathological evaluations of the liver

Part of the liver tissue of each rat was cut and fixed in 10% formalin solution. The fixed tissue was processed for light microscopy using the conventional histological procedures, such as dehydration, clearing, impregnation (infiltration), embedding, sectioning, mounting and staining. The sliced were stained with Hematoxylin and Eosin and examined under the microscope for hispathological changes. Photomicrographs of the slides were prepared.

Procedure for tissue processing

Preparation of Fixatives

FORMAL SALINE

40% formaldehyde ……..... 100ml

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Sodium chloride……. 9g

Distilled water …… 900ml

Fixation

Tissues were preserved in 10% formosaline for at least 2-4 hours to prevent autolysis and putrefaction.

Dehydration

The fixed tissue was passed through ascending grades of alcohol to remove the water component. First, the tissue cassettes were soaked in 70% alcohol for 1hour and then in 90% alcohol for 1hour 30minutes. Then, tisues were passed through 95% alcohol for 1hour 30minutes and finally, through absolute alcohol for 2hours.

Clearing

The process of clearing involves the removal of alcohol and other dehydrating agents, which are not miscible with paraffin. The clearing agents are miscible with both alcohol and paraffin and have high refractive index, which makes the tissue transparent. The common clearing agent is xylene. After dehydration, the tissues were cleared in xylene for one hour before transferring to paraffin wax.

Infiltration

After clearing, the tissues were infiltrated with paraffin wax. After clearing, the tissues were first placed in the first paraffin wax bath for 2 hrs and then in the second bath for another 2 hrs. Molten paraffin for impregnation was maintained at 2°C above the melting point of the wax in the embedding mould oven.

Embedding

The impregnated tissues were placed in tissue cassettes containing embedding medium (paraffin wax) and allowed to solidify to form tissue block. Excess paraffin wax was trimmed to size.

Technique of cutting paraffin embedded section

The tissue block was sectioned at 5µm to form ribbon using a Rotary microtome. The sections were floated on a water bath having temperature 43-47°C to remove the wrinkles.

Mounting of sections

The egg white (albumin) and glycerine were mixed together and a thin smear made on the microscope glass slide. The albuminized slides were used to pick tissue section from the water warm bath. The slides were kept at 62°C for few seconds to melt the wax. After melting the wax, the slides were put in xylene for 10 minutes to wash off the wax and then air dried for 1 minute.

Staining

The purpose of staining is to optically differentiate the tissue components by variation in colour. The tissues were stained with Haematoxylin and Eosin. The slides were first put in haematoxylin stain for 3-5 minutes and kept under running tap water for a few minutes. Then the slides were immersed in 1 % and 2 % HC1 respectively and again kept under running tap water for 30 minutes. Then the slides were dipped in eosin stain for a few seconds. The excess stain was washed off by 90% alcohol, dipped in xylene and kept for mounting.

Mounting

Mounting process is placing the mounting medium on the stained tissue section and covering it with coverslip. A drop of mounting medium, (D.P.X) was placed on the coverslip which was subsequently used in covering the tissue slide. Excess medium was wiped off. The slides were then ready for examination under microscope.

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Photomicrography

The slides were snapped with a Canon digital camera (DSC 7H SONY, U.S.A, 10 megapixels) and examined using x10 objective lens of the microscope for focusing and viewing with the x40 objective lens of the microscope for a higher magnification.

Elisa (Enzyme Linked Immunosorbent Assay)

Enzyme linked immunosorbent assay of the liver

Enzymatic assay for P-53 activities was carried out. The tissues were homogenized using spectrophotometric techniques. The tissue was placed in 0.25M sucrose solution and then in porcelain mortar, was homogenized thoroughly using the porcelain pestle. Tissue homogenate was collected in a 5ml serum bottle and was centrifuged at 3000rpm for 15 minutes using a centrifuge (Model 90-1). The supernatant was collected with Pasteur pipettes and placed in a freezer at -4^{O}C, and thereafter assayed.

Principle of P-53

The assay was done using Indirect Enzyme-Linked Immunosorbent Assay (ELISA). The micro titer plate wells were coated with antibody specific for P-53. After incubation, uncover the micro titer plate and discard the solution into a container. The coating solution was removed, and the plate was washed twice by filling the wells with 200 µl PBS. The remaining protein-binding sites in the coated wells were blocked by adding 200 µl blocking buffer and incubated for 30 minutes at room temperature to prevent false positive results. The sample containing antibody (rat monoclonal antibody) was added to the wells and the plate was incubated at 37°c. The plate was washed so that unbound antibody is removed. The secondary antibody conjugated to an enzyme (anti-rabbit IgG) was added. The plate was washed, so that unbound enzyme-linked antibodies are removed. Horseradish phosphatase substrate which was converted by the enzyme to produce a colored product. Reaction of a substrate with the enzyme to produce a colored product. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450nm. The absorbance/optical density of each well was determined using a microplate reader.

Detection range

250pg/mL-5000pg/mL

Sensitivity

The minimum detectable dose of P-53 is typically 0.1ng/mL.

Specificity

This assay has high sensitivity and excellent specificity for detection of rat P-53. No significant cross-reactivity or interference between rat P-53 and analogues was observed.

P-53 Assay Procedure

All the reagents were brought to room temperature (18-25°C) before use for 30 minutes. The sample were then centrifuged before the assay, assay layout sheet determined the number of well used, the remaining well and the desiccant were put into the pouch and sealed with the Ziploc, the unused wells were stored at 4°C. 100µl of standard and sample per well were added, covered with the adhesive strip provided, and then incubate for 2hours at 37°C.

A plate layout was provided to record standards and samples assayed; the liquid of each well was removed without washing. Biotin- antibodies (1x) of 100µl were added to each well and then covered with a new adhesive strip that were also incubated for 1hour at 37°C. It was warmed up to room temperature and mixed gently until the solution appeared uniform.

Each well was aspirated and washed; the process was repeated two times for a total of three washes. Each well was washed with wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or

autowasher, and allowed to stand for 2minutes. 100µl of HRP-avidin (1x) were added to each well, the microtiter plate was covered with a new adhesive strip and incubated for 1hour at 37°C.

The wells were washed five times and 90µl of TMB substrate were added to each well and incubated for 15-30 minutes at 37°C to protect it from light. 50µl of stop solution was added to each well and the plate were gently tapped to ensure thorough mixing. The optical density of each well was determined within 5 minutes, using a microplate reader set of 450nm.

Statistical Analyses

The statistical analyses of bodyweight of rats in each group between the end of Week One and Week five were carried out using Graph pad prism 2005 version. The statistical data acquired from the microplate Enzyme linked immunosorbent assay (ELISA) results were also analyzed using t-test statistical analyses on graph pad prism 2005 version. Comparisons between two groups were conducted using unpaired student t-test. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Statistical analyses of concentrations of P-53 (Male wistar rats)

Table 3.1: (MEAN±SEM) of Male Wistar rats

GROUP	DOSES OF DRUG/EXTRACT ADMINISTERED	p53 MEAN±SEM
1	PS	24.79±2.46
2	Single oral administration of 15mg/kg bw DMBA	37.81±0.09
3	Single intra-peritoneal injection of 1.25mg/kg bw Cadmium	45.27±3.45
4	Co-administration of 30mg/kg bw MO+ 15mg/kg bw DMBA	36.82±2.04
5	Co-administration of 10mg/kg bw MS+ 1.25mg/kg bw CD	16.14±0.56
6	15mg/kg bw DMBA + 15mg/kg bw MO	29.20±0.25
7	15mg/kg bwDMBA + 30mg/kg bw MO	24.76±4.31
8	10mg/kg DMBA + 10mg/kg bw MS	32.25±3.18
9	1.25mg/kg bw CD + 15mg/kg bw MO	15.58±4.87
10	1.25mg/kg bw CD + 30mg/kg bw MO	15.78±4.87
11	1.25mg/kg bw CD + 10mg/kg bw MS	25.46±2.39
12	15mg/kg bw DMBA) + standard doses of 5-FC, CP, AD, MM	17.45±5.24
13	1.25mg/kg bw CD + standard doses of 5-FC, CP, AD, MM	12.46±0.25
14	15mg/kg bw MO	15.21±0.37
15	30mg/kg bw MO	28.61±0.08
16	10mg/kg bw MS	34.32±3.42
17	Co-administration of 1.25mg/kg bw CD+ 30mg/kg bw MO	20.12±1.19

PS= Physiological saline, DMBA= 7,12- Dimethylbenz(a)anthracene, CD= Cadmium, MO= *Moringa olifera*, MS= *Musa sapentium*, 5-FC= 5-fluorouracil, CP= cyclo phosphatase, AD= Adramycin, MM= Mitomycin

Table 3.2: Statical analysis of DMBA treated experimental grouping

GROUP	DOSES OF DRUG/EXTRACT ADMINISTERED	p53 MEAN±SEM	P≤0.05 GROUP 1 VS GROUP 2-17	P≤0.05 GROUP 2 VS GROUP 3-17
1	PS	24.79±2.46		
2	Single oral administration of 15mg/kg bw DMBA	37.81±0.09	0.03*	
3	Co-administration of 30mg/kg bw MO+ 15mg/kg bw DMBA	36.82±2.04	0.06	0.68

4	15mg/kg bwDMBA + 30mg/kg bw MO	24.76±4.31	0.99	0.08
5	10mg/kg DMBA + 10mg/kg bw MS	32.25±3.18	0.21	0.22
6	15mg/kg bw DMBA + standard doses of 5-FC, CP, AD, MM	17.45±5.24	0.50	0.01*
7	15mg/kg bw MO	15.21±0.37	0.06	0.003*
8	30mg/kg bw MO	28.61±0.08	0.26	0.002*
9	10mg/kg bw MS	34.32±3.42	0.15	0.42

PS= Physiological saline, DMBA= 7,12- Dimethylbenz(a)anthracene, CD= Cadmium MO= *Moringaolifera*, MS= *Musa sapentium*, 5-FC= 5-fluorouracil, CP= cyclo phosphatase, AD= Adramycin, MM= Mitomycin.

Table 3.3: Statistical analysis of Cadmium treated experimental grouping

GROUP	DOSES OF DRUG/EXTRACT ADMINISTERED	p53 MEAN±SEM	P≤0.05 GROUP 1 VS GROUP 2-17	P≤0.05 GROUP 2 VS GROUP 3-17
1	PS	24.79±2.46		
2	Single intra-peritoneal injection of 1.25mg/kg bw Cadmium	45.27±3.45	0.04*	
3	Co-administration of 10mg/kg bw MS+ 1.25mg/kg bw CD	16.14±0.56	0.08	0.01*
4	1.25mg/kg bw CD + 15mg/kg bw MO	15.58±4.87	0.23	0.04*
5	1.25mg/kg bw CD + 30mg/kg bw MO	15.78±4.87	0.12	0.02*
6	1.25mg/kg bw CD + 10mg/kg bw MS	25.46±2.39	0.86	0.04*
7	1.25mg/kg bw CD + standard doses of 5-FC, CP, AD, MM	12.46±0.25	0.44	0.01*
8	15mg/kg bw MO	15.21±0.37	0.06	0.01*
9	30mg/kg bw MO	28.61±0.08	0.26	0.04*
10	10mg/kg bw MS	34.32±3.42	0.15	0.15
11	Co-administration of 1.25mg/kg bw CD+ 30mg/kg bw MO	20.12±1.19	0.23	0.02*

The results presented in Table 3.2 indicate statistically significant mean values (P≤0.05) of P-53 concentrations in rats of group 2 (P=0.03), when compared with Control Group 1 (24.79±2.46) as presented in (Table 3.2) of DMBA treated groups. Results also showed statistically significant mean values (P≤0.05) of P-53 concentrations (pg/ml) in rats of group 6 (P=0.01), group 7 (0.003) and group 8 (0.002) when compared to group 2 (37.81±0.09) as presented in (Table 3.2) .

Table 3.3 also indicate statistically significant mean values (P≤0.05) of P-53 concentrations in rats of group 2 (P=0.04), when compared with Control Group 1 (24.79±2.46) as presented in (Table 3.3) of cadmium treated groups above. (Table 3.3) also indicate statistically significant mean values (P≤0.05) of P-53 concentrations (pg/ml) in rats of group 3 (P=0.01), group 4 (0.04), group 5 (0.02), group 6 (P=0.04), group 7 (P=0.01), group 8 (0.01), group 9 (0.04) and group 11 (P=0.02) when compared to group 2 (37.81±0.09) as presented in (Table 3.3) of cadmium treated groups.This implied that DCMB and cadmium-induced hepatotoxicity led to upregulation of P-53 and desensitization resulting in tumour suppression in rats of experimental groups (Table 3.2 and 3.3).

This observation is in agreement with the observation of a study by (Lee *et al*., 2006), which investigated the process of sensitization and tumour suppression in mice that have a specific p53 mutant to retain the ability to induce apoptosis, allowing them to efficiently suppress oncogen induced tumours, thus suggesting that the proapoptotic function of p53 may play an important role in its antitumor effects.

Furthermore, statistical analyses of p53 concentrations presented in Table 3.2 and 3.3 implied that the ethanol extract of the leaves of *Moringa oleifera and Musa sapentium* ameliorated DMBA, cadmium-induced increased desensitization via downregulation and positive immunomodulation of p53. However, results also showed statistically non-significant lower mean values ($P>0.05$).

CONCLUSION

Extracts of Moringa oleifera and Musa sapentium ameliorated desensitization in DMBA(7,12-dimethylbenz(a)anthracene) and cadmium-induced tumour suppressor protein and possibly possess sensitization potentials. Furthermore, the extracts also ameliorated increased proliferation in DMBA and cadmium-induced proliferation and possibly possess anti-proliferation potentials in male rats.

Limitations Of The Study

The study focused on p53 as a single molecular biomarker, without assessing downstream apoptotic or cell cycle regulatory proteins that could further clarify mechanistic pathways. Biochemical liver function markers such as serum ALT, AST, and ALP were not included. Also, the study utilized crude plant extracts, and specific bioactive compounds responsible for the observed effects were not isolated or characterized. Finally, the investigation was limited to male Wistar rats, and sex-dependent responses were not explored.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

1. Future studies should include additional molecular markers related to apoptosis, oxidative stress, and inflammation to further elucidate the mechanisms underlying hepatoprotection.
2. Biochemical liver enzyme assays should be incorporated to strengthen the assessment of liver function.
3. Fractionation and isolation of active phytochemical constituents of Moringa oleifera and Musa sapentium are recommended to identify specific compounds responsible for the protective effects.
4. Long-term and dose-response studies should be conducted to establish safety margins and therapeutic relevance.
5. Inclusion of both male and female animal models may provide insight into possible sex-related differences in response.

REFERENCES

1. Adefisan-Adeoye, A. O., Ayanbanjo, O. O., Adeoye, T. D., Jayesimi, T. E., Unuofin, J. O., Lebelo, S. L., & Adaramoye, O. A. (2025). Bisdemethoxycurcumin chemoprevents 7,12-dimethylbenz(a)anthracene-induced mammary toxicity via modulation of oxidative processes. Scientific Reports, 15, 9170. https://doi.org/10.1038/s41598-025-94168-x
2. Adegbola, P. I., & Adetutu, A. (2024). Genetic and epigenetic modulations in toxicity: The two-sided roles of heavy metals and polycyclic aromatic hydrocarbons from the environment. Toxicology Reports, 12, 502–519. https://doi.org/10.1016/j.toxrep.2024.04.010
3. Adeoye, S. C., et al. (2023). p53 gene expression and nitric oxide levels after artemisinin-caffeine treatment in breast, lungs and liver of DMBA-induced tumorigenesis. Asian Pacific Journal of Cancer Prevention, 24(2), 451–458. https://journal.waocp.org/article_90476.html
4. Aremu, A. O., Luo, B., & Mussarat, S. (2024). Medical ethnobotany: Insights into indigenous knowledge, medicinal plants, and pharmacological relevance. BMC Complementary Medicine and Therapies, 24, Article 216. https://doi.org/10.1186/s12906-024-04515-0
5. Badawi, K., El Sharazly, B. M., Negm, O., Khan, R., & Carter, W. G. (2024). Is cadmium genotoxicity due to the induction of redox stress and inflammation? A systematic review. Antioxidants, 13(8), 932. https://doi.org/10.3390/antiox13080932

6. Chen, Y., Mei, Y.-Q., Hou, L., & Li, K.-J. (2025). Therapeutic potential of plant-derived natural products against drug-induced liver injury. Frontiers in Pharmacology, 16, 1652860. https://doi.org/10.3389/fphar.2025.1652860
7. Etukudo, E. M., Usman, I. M., Oviosun, A., Ojiakor, V. O., Jama, I. A., Makena, W., Makeri, D., Owembabazi, E., Aja, P. M., & Anyanwu, E. (2025). Exploring the phytochemical profile, antioxidant and anti-inflammatory potential of Bidens pilosa: A systematic review. Frontiers in Pharmacology, 16, 1569527. https://doi.org/10.3389/fphar.2025.1569527
8. Farahat, Y. A., El-Sayed, N. M., Hazem, R. M., Mehanna, E. T., & Radwan, A. (2025). Sinapic acid ameliorates cadmium-induced hepatotoxicity: Modulation of oxidative stress, inflammation, and apoptosis. Biomedicines, 13(5), 1065. https://doi.org/10.3390/biomedicines13051065
9. Fatima, G., Khan, S., Shukla, V., Awaida, W., & Li, D. (2025). Nutraceutical formulations and natural compounds for the management of chronic diseases. Frontiers in Nutrition, 12, Article 1682590. https://doi.org/10.3389/fnut.2025.1682590
10. Gervásio, S. V., & Batitucci, M. C. P. (2023). Biological, antioxidant and phytochemical activities of Musa spp. Ciência Rural, 53(12), e20220636. https://doi.org/10.1590/0103 8478cr20220636
11. Martínez-Sena, T., Moro, E., Moreno-Torres, M., Quintás, G., Hengstler, J. G., & Castell, J. V. (2023). Metabolomics-based strategy to assess drug hepatotoxicity and uncover the mechanisms of hepatotoxicity involved. Archives of Toxicology, 97(6), 1723–1738. https://doi.org/10.1007/s00204-023-03474-8
12. Mugale, M., Dev, K., More, B. S., & Singh, I. (2024). A comprehensive review on preclinical safety and toxicity of medicinal plants. Clinical Complementary Medicine and Pharmacology, 4, 100129. https://doi.org/10.1016/j.ccmp.2024.100129
13. Su, X., Lu, G., Ye, L., Shi, R., Zhu, M., Yu, X., Li, Z., Jia, X., & Feng, L. (2023). Moringa oleifera Lam.: A comprehensive review on active components, health benefits and application. RSC Advances, 13(35), 24353–24384. https://doi.org/10.1039/D3RA03584K
14. Wang, C., Kang, H., Yi, Y., et al. (2023). Rictor mediates p53 deactivation to facilitate the malignant transformation of hepatocytes and promote hepatocarcinogenesis. Journal of Translational Medicine, 21(919). https://doi.org/10.1186/s12967-023-04799-9
15. Wang, C., Wang, X., Deng, Y., & Hu, L. (2025). Network toxicology combined with molecular docking technology to explore the molecular mechanism of amatoxin causing liver injury. Scientific Reports, 15, 26068. https://doi.org/10.1038/s41598-025-11720-5
16. Wang, L., Shao, Z., Wang, X., Lu, W., & Sun, H. (2025). Xenobiotic-induced liver injury: Molecular mechanisms and disease progression. Ecotoxicology and Environmental Safety, 303, 118854. https://doi.org/10.1016/j.ecoenv.2025.118854
17. Wittke, C. I., Cheung, E. C., Athineos, D., Clements, N., Butler, L., Hughes, M., Morrison, V., & Watt, D. M. (2025). p53 and TIGAR promote redox control to protect against metabolic dysfunction-associated steatohepatitis. JHEP Reports, 7(7), 101397. https://doi.org/10.1016/j.jhepr.2025.101397
18. Yadav, R., Kumar, P., Amit, et al. (2024). Synergistic effects of whole plant extracts: A comparative study with isolated bioactive compounds. African Journal of Biological Sciences, 6(15), 7988–8011. https://doi.org/10.48047/AFJBS.6.15.2024.7988-8011
19. Zhao, Y., Liu, Y., & Zhang, S. (2024). Unraveling the guardian: p53's multifaceted role in the DNA damage response and tumor suppression. International Journal of Molecular Sciences, 25(23), 12928. https://doi.org/10.3390/ijms252312928