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PLUMBAGIN AND GRISEOFULVIN MITIGATE NDEA-INDUCED HEPATIC DAMAGE IN MICE

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ABSTRACT

Background Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality globally. N-nitrosodiethylamine (NDEA), a potent environmental carcinogen, plays a key role in liver tumorigenesis. Natural compounds such as plumbagin and griseofulvin have demonstrated anticancer properties in various preclinical models but remain underexplored in liver cancer. **Objective** This study investigates the protective effects of plumbagin and griseofulvin against NDEA-induced hepatic toxicity and genotoxicity in Swiss albino mice, using sorafenib as a reference compound. **Methods** Male Swiss albino mice were randomly divided into seven groups (n=6). Acute hepatotoxicity was induced via oral NDEA (200 mg/kg), followed by treatment with low and high doses of plumbagin (2 and 6 mg/kg, i.p.), griseofulvin (225 and 450 mg/kg, oral), and sorafenib (30 mg/kg, oral). Morphological, behavioral, physiological, and genomic parameters were monitored. DNA damage was evaluated using the alkaline Comet assay. **Results** NDEA exposure led to significant body weight loss, liver enlargement, behavioral alterations, and severe genotoxicity. Treatment with plumbagin and griseofulvin reversed these effects in a dose-dependent manner. High doses of both compounds significantly reduced tail DNA percentage, tail moment, and olive tail moment ($p < 0.05$), comparable to or exceeding sorafenib efficacy. **Conclusion** Plumbagin and griseofulvin exert genoprotective and hepatorestorative effects in NDEA-induced liver toxicity. These findings support their potential as anticancer agent for liver cancer.

KEYWORDS: Hepatocellular carcinoma, Genotoxicity and hepatoprotection, N nitrosodiethylamine (NDEA).

INTRODUCTION

Cancer remains a leading global health challenge, driven by both genetic alterations and environmental exposures. It arises from disruptions in cellular homeostasis, often due to dysregulated cell cycle control and activation of oncogenic pathways that enable cells to proliferate uncontrollably and evade normal checkpoints.[1,2] According to the World Health Organization (2022), approximately 20 million new cancer cases and 9.7 million deaths were reported globally, with projections estimating 4.8 million additional cases by 2050.[3,4] Liver cancer is the third leading cause of cancer-related deaths worldwide; with hepatocellular carcinoma (HCC) accounting for the majority of cases.[5] Its incidence is particularly high in Asia, largely due to chronic hepatitis B and C infections, and increasingly due to non-alcoholic fatty liver disease (NAFLD) in developed countries.[6,7] Despite advancements in therapy, current treatment options for HCC remain limited in efficacy, necessitating the identification of new therapeutic agents.[4]

Environmental carcinogens such as N-nitrosodiethylamine (NDEA) significantly contribute to liver carcinogenesis. NDEA, a potent hepatotoxin, is present in various sources including tobacco products, preserved foods, betel leaves, and industrial waste.[8,9] It undergoes metabolic activation via cytochrome P450 enzymes, producing reactive metabolites that induce oxidative stress, DNA alkylation, and chromosomal instability, thereby initiating hepatocarcinogenesis.[10]

Given the genotoxic impact of NDEA, research has turned to natural bioactive compounds for safer therapeutic alternatives. Plumbagin ($C_{11}H_8O_3$), a naphthoquinone derivative found in Plumbago species, exhibits anti-cancer effects by inhibiting VEGFR2-mediated angiogenesis and inducing oxidative stress in tumor cells.[11] It has demonstrated efficacy across various cancers including colon, prostate, and ovarian models.[12,13]

Griseofulvin ($C_{17}H_{17}ClO_6$), a fungal metabolite traditionally used as an antifungal, has also gained attention in oncology. It disrupts microtubule dynamics during mitosis, arresting cancer cell division and promoting apoptosis.[14] Besides *Penicillium griseofulvum*, it is also derived from *Xylaria flabelliformis* and other fungi.[15] Its favorable safety profile and multifaceted mechanisms suggest potential for repurposing in liver cancer therapy.[16]

This study investigates the protective potential of plumbagin and griseofulvin against NDEA-induced genotoxicity in mouse liver tissue, using the alkaline Comet assay as a sensitive biomarker for DNA strand breaks. Comparative evaluation with sorafenib, a standard HCC treatment, provides insights into the efficacy of these natural compounds as adjunct or alternative therapeutic agents.

MATERIALS AND METHOD

Chemicals and reagents

N-Nitrosodiethylamine (NDEA) was purchase from Sigma Aldrich, a company based in the USA. Sorafenib (200 mg) tablets were obtained from Cipla Company. Assay kits for various parameters were purchased from Accurex Biomedical Pvt. Ltd. All the chemicals and reagents used in this study were of high quality and purchased from reputable companies such as HIMEDIA, MERCK, and SRL, ensuring the highest available purity.

Selection of experimental animals

Healthy adult male Swiss albino mice (Mus musculus), weighing 25–35 g, were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, India. Animals were housed in polypropylene cages under standard laboratory conditions with ad libitum access to a pellet diet and water.

Swiss albino mice were chosen for their genetic stability, ease of handling, high reproductive rate, and well-established role in toxicological and pharmacological research. Their physiological and genetic resemblance to humans, along with minimal hormonal variability and cost-effectiveness, make them a reliable model for *in vivo* studies.

Ethical Considerations and Animal Procurement

All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of Banasthali Vidyapith, Rajasthan, India, under reference number BV/IAEC/2023/4824, in accordance with CPCSEA guidelines for the care and use of laboratory animals.

Male Swiss albino mice (Mus musculus), initially weighing 12–15 g, were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India. Prior to experimentation, animals were acclimatized for 15 days under standard laboratory conditions in the animal facility of the Department of Bioscience and Biotechnology,

Banasthali Vidyapith. Experimental procedures were initiated once the mice reached a body weight of 25–30 g.Maintenance monitoring and experimental design of animals.

A total of 42 healthy adult male Swiss albino mice were evenly distributed into seven experimental groups. The mice were housed in the animal facility of the Department of Bioscience and Biotechnology, Banasthali University, under controlled environmental conditions: Temperature: 23 ± 3°C, Relative Humidity: 50 ± 16%, Light/Dark Cycle: 12 hours.

The mice were kept in well-ventilated polypropylene cages with stainless steel bar lids, with six mice per cage. To maintain hygiene, cages were regularly cleaned using standard detergents and disinfected with an alcohol-based solution. The entire animal house routinely sterilized with disinfectants to ensure a pathogen-free environment. All mice received a pelleted diet (rice bran: basal feed; 1:1) and had ad libitum access to water throughout the study. The total experimental duration was 28 days.

Dosage and Treatment Strategy

The hepatocarcinogen N-nitrosodiethylamine (NDEA) was administered orally at a dose of 200 mg/kg body weight on Day 21 of the study to induce liver toxicity, as per established protocols.[17] Experimental animals were then treated with natural and standard compounds at dose levels derived from prior toxicity and efficacy studies.

Griseofulvin was administered orally at 225 mg/kg (low dose) and 450 mg/kg (high dose) once daily for 14 consecutive days.[18] Plumbagin was given intraperitoneally at 2 mg/kg (low dose) and 6 mg/kg (high dose) once daily for 14 days.[19] The standard reference drug, Sorafenib, was administered orally at 30 mg/kg and 400 mg/kg body weight for 14 days, based on previously reported LD_{50} values and pharmacological evaluations.[20,21,22]

A total of 42 male Swiss albino mice were randomly divided into seven groups (n = 6) and treated over a 28-day period:

Group I (Control): Received normal saline orally for 28 days.

Group II (DENA control): Received a single oral dose of NDEA (200 mg/kg b.w.) on Day 21.

Groups III & IV (Plumbagin-treated): Pre-treated with NDEA (single dose, Day 14), followed by Plumbagin administration at low (2 mg/kg) and high (6 mg/kg) doses intraperitoneally for the next 14 days.

Groups V & VI (Griseofulvin-treated): Pre-treated with NDEA (single dose, Day 14), followed by oral Griseofulvin treatment at low (225 mg/kg) and high (450 mg/kg) doses for 14 days.

Group VII (Sorafenib-treated): Pre-treated with NDEA, followed by oral administration of Sorafenib (30 mg/kg or 400 mg/kg) for 14 days.

Organ Isolation

At the end of the treatment period, animals were sacrificed for sample collection and downstream analyses.[23,24]

On Day 29, all animals were humanely euthanized via cervical dislocation following ethical guidelines. The liver was carefully excised, rinsed with ice-cold phosphate-buffered saline (PBS; 0.1 M, pH 7.4, 1:9) to remove residual blood, and examined for macroscopic abnormalities such as nodular formations. The liver weight was recorded for each animal and used for subsequent biochemical and genotoxic analyses.

Body weight and organ weight assessment

Body weight of all animals was recorded at the beginning and end of the experimental period using a precision digital balance. Organ weights, specifically the liver, were measured post-sacrifice using a digital analytical balance.

$$Relative\ organ\ weight = \frac{organ\ weight\,(g)}{body\ weight\ of\ mice\ on\ sacrified\ day(g)} \times 100$$

$$Body\ weight\,(\%) = \frac{final\ body\ weight - Initial\ body\ weight}{initial\ body\ weight} \times 100$$

DNA Damage Assessment in Liver Tissue Using the Comet Assay

Assessment of DNA damage in liver tissue was carried out using the alkaline Comet assay, following modifications adapted from previously reported methodologies.[25,26] Freshly isolated liver samples were rinsed with chilled RPMI-1640 medium containing 15% DMSO and 1.8% NaCl. The tissue was finely chopped in the same medium to obtain a uniform single-cell suspension. After allowing coarse fragments to settle, the supernatant with

liberated cells was collected, centrifuged at 1000 rpm for 5 minutes at 4°C, and the resulting pellet was resuspended in 0.5 mL of fresh medium.

An aliquot of 100 µL from this suspension was mixed with 1% low melting point agarose and spread onto microscope slides pre-coated with 1.5% normal agarose. These slides were allowed to gel at 4°C for 5-15 minutes. Cell lysis was performed in the dark at low temperature using a lysis buffer (pH 10) containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100, and 10% DMSO, for 50 minutes.

Post-lysis, the slides were rinsed with distilled water and immersed in alkaline electrophoresis buffer for 25 minutes to facilitate DNA unwinding. Electrophoresis was carried out at 25 V and 300 mA for 25 minutes. Slides were then neutralized using 0.4 M Tris buffer (pH 7.5) and washed with methanol. DNA was stained using 2.5 µg/mL propidium iodide for 15 minutes in the dark to prevent additional damage.

Slides were visualized under a fluorescence microscope equipped with an image analysis system. For each treatment group, 50 randomly selected nuclei were evaluated. Intact nuclei indicated undamaged DNA, while comet-shaped nuclei indicated strand breaks. The extent of DNA damage was quantified using three key parameters: percentage of DNA in the tail, tail moment, and olive tail moment.

Statistical analysis

Data were analyzed to determine statistical significance using the Student's t-test and two-way analysis of variance (ANOVA) via GraphPad Prism software. A p-value of less than 0.05 was considered statistically significant. Results are presented as mean ± standard deviation (SD), based on two or three independent experimental replicates, where applicable.

RESULTS

Acute Toxicity Study of Compounds

The selection of compound doses in this study was selected by prior experimental evidence demonstrating both efficacy and safety. According to Kim et al., 2011, griseofulvin (GF) was administered orally to BALB/c mice at a daily dose of 450 µg per animal following subcutaneous injection of MPC11 murine myeloma cells. This study significantly suppressed tumor growth and prolonged survival without observable adverse effects, indicating low acute toxicity and high therapeutic potential.

Similarly, [7]Yang et al., 2020, investigated the anti-tumor effects of plumbagin in BALB/c mice using intraperitoneal doses of 2 mg/kg, 4 mg/kg, and 6 mg/kg over six weeks. The highest dose achieved a tumor inhibition rate of 50.32%, while lower doses exhibited dose-dependent anti-tumor activity with no significant signs of toxicity. For hepatocellular carcinoma (HCC), Zakaria et al., 2022[17], employed diethylnitrosamine (DENA) at a dose of 200 mg/kg administered intraperitoneally in drinking water for 16 weeks. Following induction, sorafenib was administered orally at 30 mg/kg/day for four weeks to evaluate its therapeutic efficacy. These prior findings provided a rational basis for selecting the respective compound dosages used in the present study.

Morphological and Behavioral Changes

Untreated control animals (Group I) exhibited no observable functional or inflammatory abnormalities and maintained normal physiological behavior, including consistent grip strength, locomotor activity, sleep patterns, eye clarity, salivation, urination, and fecal output. By day 28 of NDEA administration, all mice in Group II displayed prominent signs of toxicity, including skin discoloration, barbered fur, and the development of subcutaneous lesions (Figure 1B). In the treated groups (III to VII), mild skin discoloration was observed earlier, beginning around day 14 (Figures 1C-F).

NDEA exposure also led to behavioral changes, such as impaired locomotion, ear retraction suggestive of pain or distress, and a reduction in grip strength and tail reflexes (Supplementary Table 1; Figure 1A-F). Groups III to VII, despite receiving therapeutic interventions, still showed signs of behavioral impairment by day 14, including reduced food intake, muscle tone, and responsiveness to vocal and tactile cues. Increased urination was also observed at this stage (Supplementary Table 1).

From day 14 onwards, additional symptoms appeared, including skin rashes, tremors, heightened hyperactivity, and altered vocal behavior. These behavioral patterns suggest neurological involvement and systemic stress responses.

Changes in body weight, a sensitive indicator in toxicological studies, were consistent with systemic toxicity. These weight fluctuations reflect disturbances in energy metabolism and alterations in body composition, in line with established toxicological findings.[27]

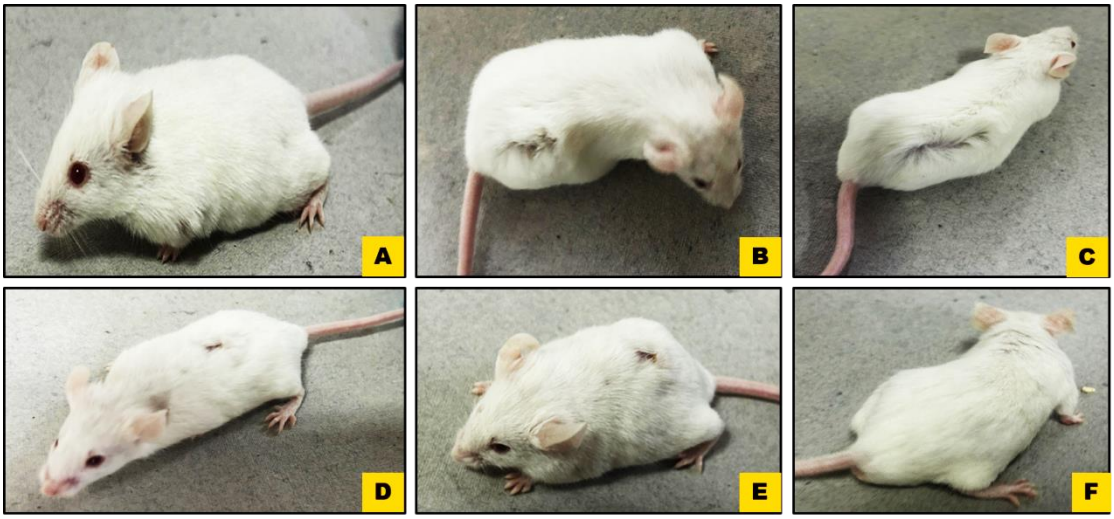

Figure 1. Morphological and behavioural changes;- Clinical signs and lesions post-NDEA administration (A) Control mice (B) Mild fur barbering (Day 28th) (C) Plain fur barbering (D) Visible lesions (E) Abnormal movement during dosing (F) Ears retracted, indicating distress.

Macroscopic Hepatic Morphological Features

Gross morphological examination of liver tissues across experimental groups revealed significant alterations induced by NDEA and therapeutic effects following treatment with anticancer compounds.

Group II (NDEA-exposed) exhibited marked hepatic enlargement and surface irregularities, including multiple tumor nodules and pale discoloration hallmarks of advanced hepatic damage and carcinogenesis (Figure 2.1D-F). In contrast, livers from the control group (Group I) maintained a smooth, uniform surface with no visible abnormalities, reflecting normal hepatic morphology (Figure 2.1A-C).

Post-treatment changes were evident in Groups III to VII. Group III (Griseofulvin-Low) and Group IV (Griseofulvin-High) showed moderate morphological improvements, with fewer nodules and reduced hepatic edema (Figure 2.2A-F). Similarly, Group V (Plumbagin-Low) and Group VI (Plumbagin-High) demonstrated substantial restoration of liver morphology, with near-complete absence of tumor nodules and visibly smoother liver surfaces (Figure 2.3A-F).

Group VII, treated with the standard drug sorafenib, exhibited the most pronounced therapeutic response. Livers appeared nearly indistinguishable from those in the control

group, with restored architecture and absence of visible lesions, underscoring sorafenib's efficacy in reversing NDEA-induced hepatic alterations (Figure 2.4A-C).

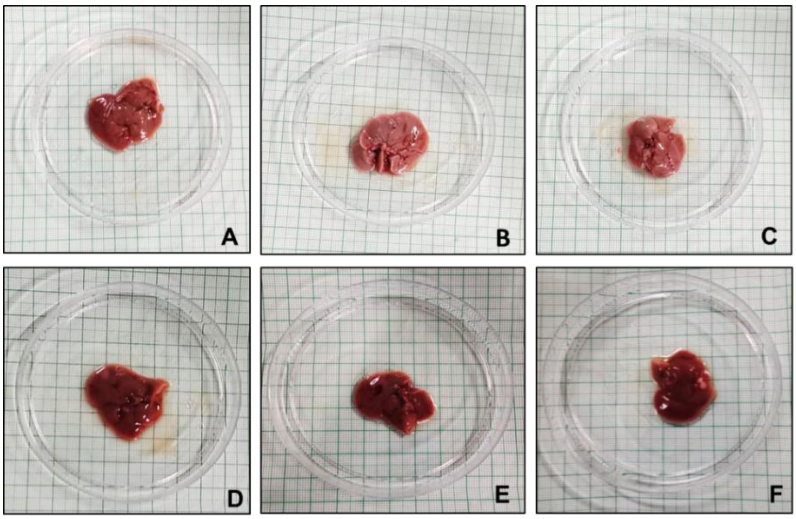

Figure 2.1. Macroscopic representative images of livers isolated from animals, illustrating hepatic morphological features: (A-C) for Group I (Control); (D-F) for Group II (Carcinogen).

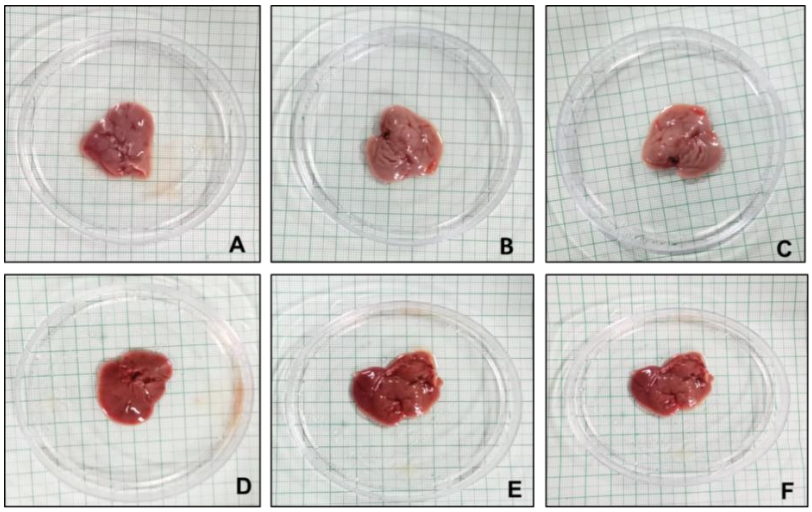

Figure 2.2. Macroscopic representative images of livers isolated from animals, illustrating hepatic morphological features: (A-C) for Group III (Griseo-L); (D-F) for Group IV (Griseo-H).

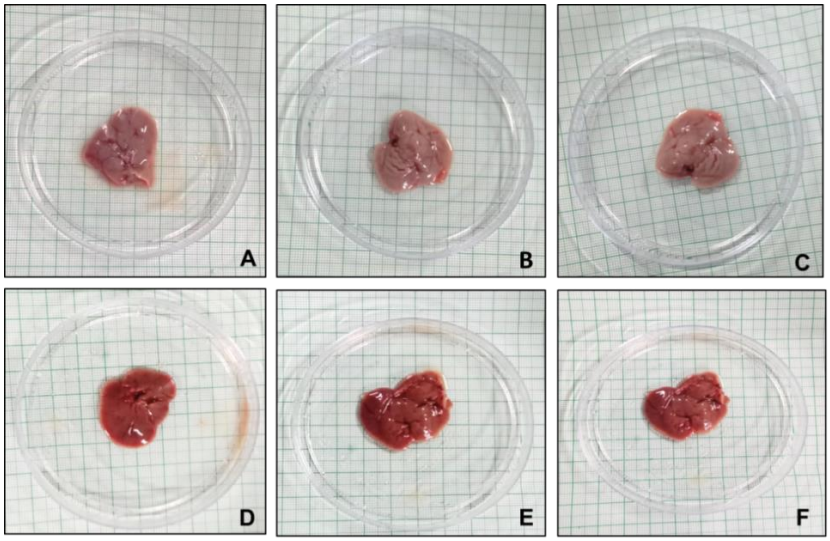

Figure 2.3. Macroscopic representative images of livers isolated from animals, illustrating hepatic morphological features: (A-C) for Group V (Plum-L); (D-F) for Group VI (Plum-H).

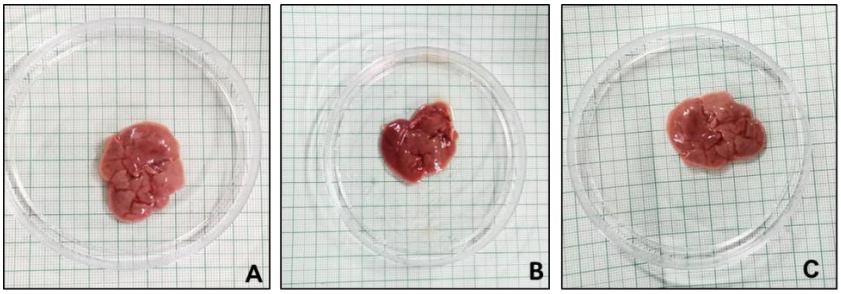

Figure 2.4. Macroscopic representative images of livers isolated from Group VII (Standard) animals, illustrating hepatic morphological features: (A-C).

Effect of NDEA, Anticancer Compounds, and Sorafenib on Body and Organ Weight

The impact of NDEA, tested anticancer compounds, and sorafenib on body and liver weights was assessed by recording initial and final body weights of all mice. Mean values and relative organ weights are summarized in Table 1 and Figure 3A-B.

Mice in Group II (NDEA-exposed) showed a significant reduction in body weight (22.55 ± 0.09 g, $p < 0.001$) compared to the normal control group (36.55 ± 0.30 g). Post-treatment groups (III–VII) demonstrated significantly increased final body weights of 27.77 ± 0.28, 29.22 ± 0.30, 27.33 ± 0.95, 28.44 ± 0.30, and 31.44 ± 0.25 g, respectively, compared to the carcinogen-exposed group II.

Absolute liver weight was significantly elevated in the NDEA group (3.63 ± 0.48 g, $p < 0.001$) relative to controls (2.09 ± 0.29 g). Treatment with the anticancer compounds (both low and high doses) and sorafenib significantly reduced liver weights ($p < 0.001$ and $p < 0.05$) compared to group II (Table 1), indicating a protective effect.

Table 1. Variation in final body weight, absolute and relative organ weights in liver cancer-bearing animals.

Groups	Body weight (gm)		Organ weight	
	InitialBW	FinalBW	Absolute organ weight	Relative organ weight (%)
Control	24.33 ± 0.54	36.55 ± 0.30	2.09 ± 0.29	4.78
Crg	25.33 ± 0.27	22.55 ± 0.09^{a}	3.53 ± 0.48^{a}	14.07
Crg+GL	23.77 ± 1.09	27.77 ± 0.28^{cd}	3.47 ± 0.59^{dc}	7.32
Crg+GH	25.22 ± 0.57	29.22 ± 0.30^{d}	3.30 ± 0.25^{b}	6.15
Crg+PL	23.11 ± 0.30	27.33 ± 0.95^{bc}	3.19 ± 0.02^{c}	6.01
Crg+PH	26.33 ± 0.54	28.44 ± 0.30^{d}	2.94 ± 0.03^{a}	5.85
Sorafenib	25.33 ± 0.96	31.44 ± 0.25^{c}	1.62 ± 0.03^{bc}	5.32

The values represent the means of triplicates, mean± standard deviation (SD). InitialBW: initial body weight; FinalBW: final body weight. Compared with control group, ^{ab}($p<0.001$ and 0.05); compared with carcinogen group (Crg), ^{cd}($p<0.001$ and 0.05); carcinogen+ Grisepfulvin low dose (crg+GL), carcinogen+ Grisepfulvin high dose (crg+GH), carcinogen+ Plumbagin low dose (crg+PL), carcinogen+ Plumbagin high dose (crg+PH).

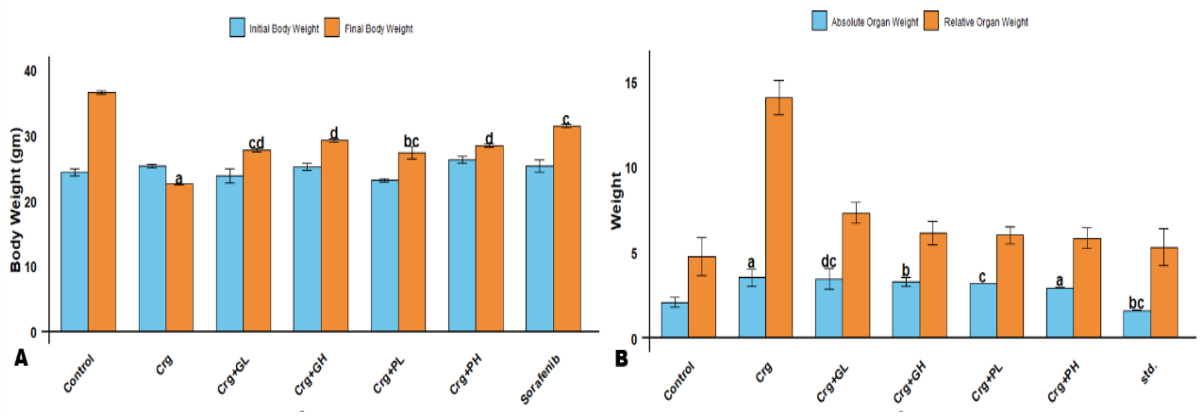

Figure 3. (A) Body weight of mice during the treatment period, normalized to the starting weight for each group (B) Liver weight analysis of mice. The values represent the means of triplicates, mean± standard deviation (SD) a($p< 0.05$) compared to control; b($p<0.01$) compared to control; c($p<0.05$) compared to carcinogenic control; d($p<0.01$) compared to carcinogenic control.

Abbreviations: Crg (carcinogenic control), Crg+GL (griseofulvin low dose), Crg+GH (griseofulvin high dose), Crg+PL (plumbagin low dose), Crg+PH (plumbagin high dose), Normal control group, Sorafenib (Std), InitialBW (initial body weight), FinalBW (final body weight), AbsoluteOW (absolute organ weight), and RelativeOW (relative organ weight).

Effect of DENA and Test Compounds on Hepatic DNA Damage

DNA damage in liver tissue was assessed by alkaline Comet assay, and quantified using % DNA in tail, tail moment, and olive tail moment parameters (Figure 4.1. and 4.2. A-C). DENA administration significantly increased genotoxicity compared to the control group ($p < 0.05$), as reflected by elevated values in % DNA tail (113 ± 4 vs. 73 ± 5), tail moment (73 ± 0.79 vs. 33 ± 0.29), and olive tail moment (85 ± 0.79 vs. 38 ± 0.69).

Treatment with Griseofulvin (GL, GH) and Plumbagin (PL, PH) demonstrated a dose-dependent reduction in DNA damage. High-dose Griseofulvin (GH) and Plumbagin (PH) groups showed notable decreases in % DNA tail (99 ± 4 and 95 ± 4), tail moment (55 ± 0.119 and 51 ± 0.133), and olive tail moment (75 ± 0.69 and 64 ± 0.93), respectively. These reductions were statistically significant compared to DENA alone ($p < 0.05$), indicating their genoprotective potential. Sorafenib, used as a reference drug, also attenuated DNA damage (83 ± 6; 41 ± 0.144; 46 ± 0.84), although to a lesser extent than high-dose test compounds. Overall, the data support the protective role of both Griseofulvin and Plumbagin in mitigating DENA-induced genotoxic effects in hepatic tissue.

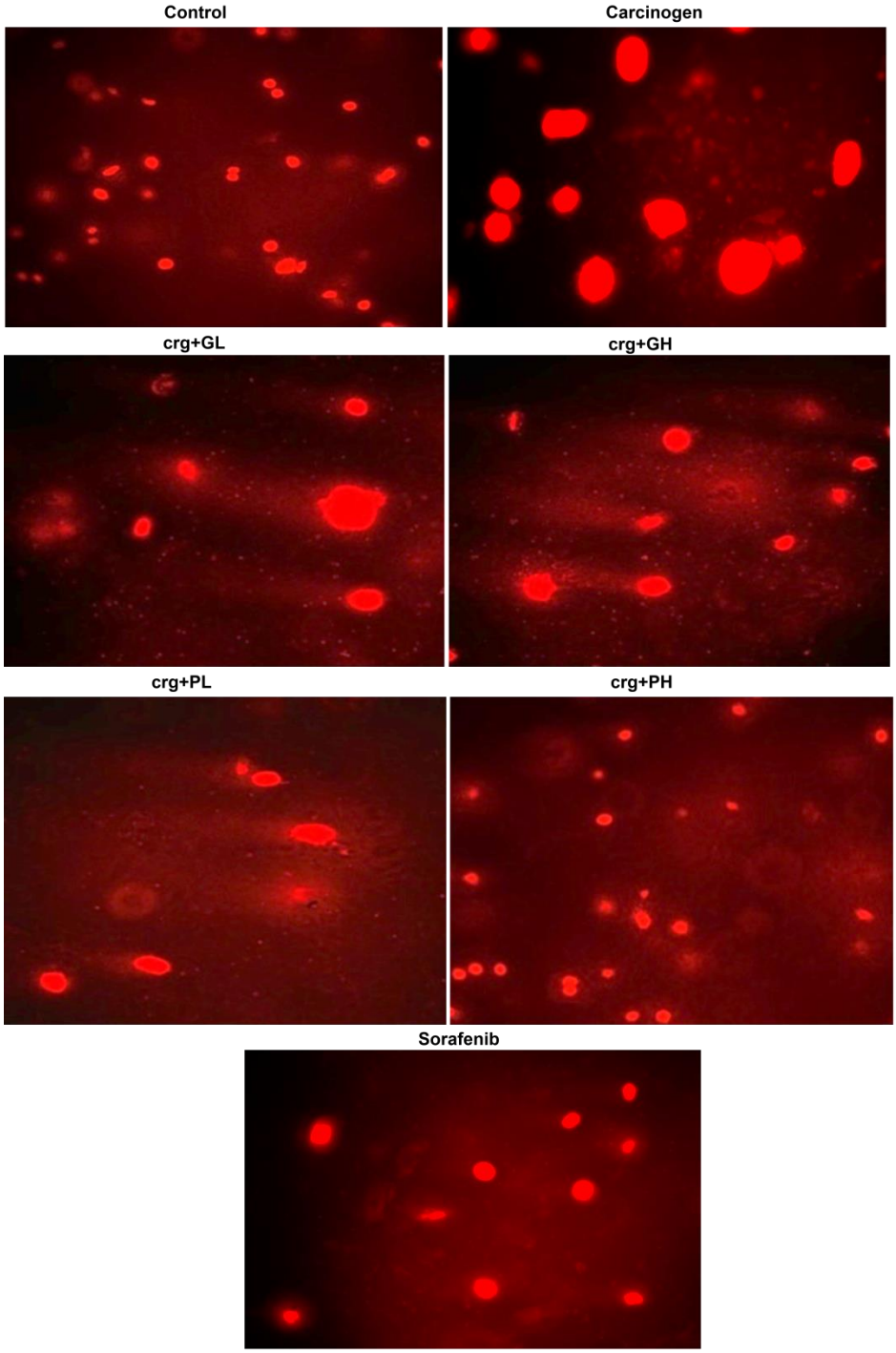

Figure 4.1 Photomicrographs of DNA migration patterns in liver tissue: Control, Carcinogen (Crg), Carcinogen + Griseofulvin Low Dose (Crg+GL), Carcinogen + Griseofulvin High Dose (Crg+GH), Carcinogen + Plumbagin Low Dose (Crg+PL), Carcinogen + Plumbagin High Dose (Crg+PH), and Sorafenib (Standard Treatment).

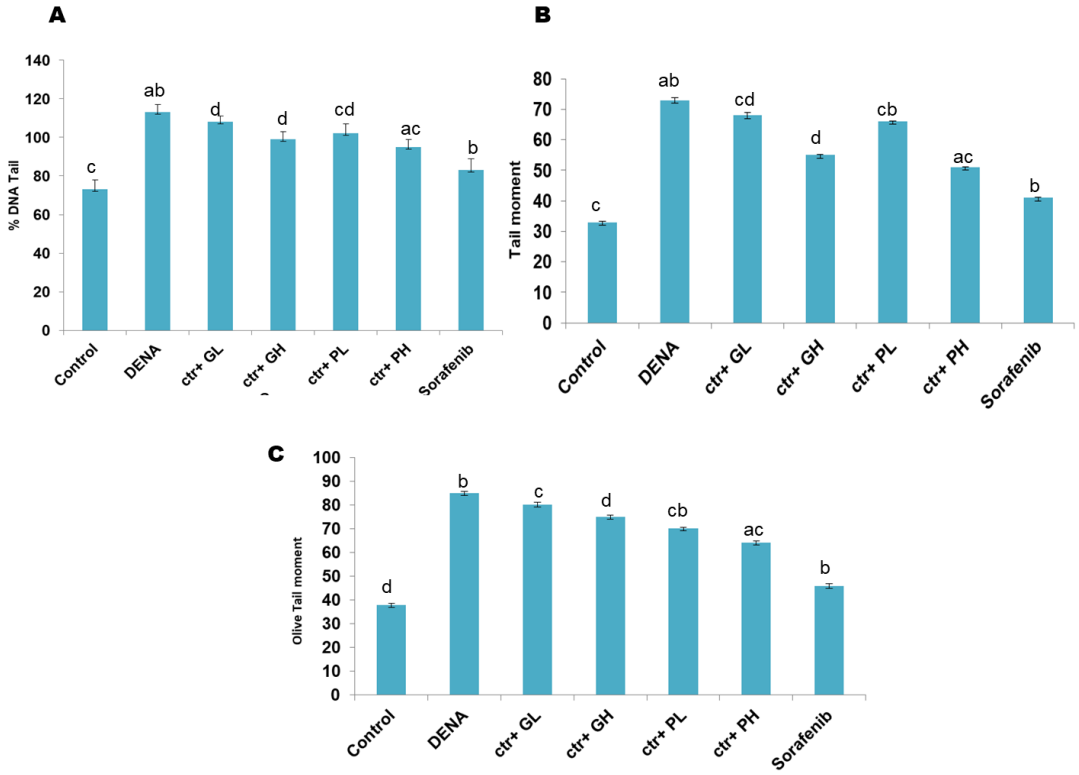

Figure 4.2 (A) % DNA in tail, (B) Tail moment, and (C) Olive tail moment. Values are presented as mean ± SEM. [ab](p < 0.001 and 0.05) compared with the carcinogen group (Crg); [cd](p < 0.001 and 0.05).

DISCUSSION

Liver cancer remains a major oncological burden, particularly in regions with high exposure to environmental carcinogens like NDEA. In this study, NDEA administration led to marked hepatotoxicity and genotoxicity, as evidenced by gross morphological changes, behavioral disturbances, increased liver weight, and DNA fragmentation in hepatocytes. These findings align with the established pathophysiological mechanisms of NDEA, which involve cytochrome P450-mediated metabolic activation and generation of reactive oxygen species, leading to oxidative DNA damage and chromosomal instability.

The observed behavioral changes ear retraction, impaired locomotion, and skin lesions further support systemic toxicity and stress, likely due to hepatic metabolic failure and neuroinflammation. Importantly, treatment with plumbagin and griseofulvin demonstrated dose-dependent mitigation of both morphological and genomic damage. These effects are consistent with prior reports highlighting plumbagin's pro-oxidative cytotoxicity toward tumor cells through inhibition of VEGFR2-mediated angiogenesis, and griseofulvin's disruption of microtubule dynamics and induction of mitotic arrest.

Compared to sorafenib, which is currently used as a standard HCC therapy, the natural compounds showed similar or superior reduction in DNA damage markers. While sorafenib attenuated genotoxicity, its protective effect was less pronounced than that of high-dose plumbagin or griseofulvin, suggesting that natural compounds could serve as valuable complementary agents in HCC treatment regimens. The liver morphology of treated groups showed remarkable restoration, particularly in the high-dose plumbagin and sorafenib groups, reinforcing the therapeutic relevance of these compounds. Overall, the results suggest a strong chemopreventive potential of these agents against carcinogen-induced hepatic damage.

CONCLUSION AND FUTURE PERSPECTIVES

This study demonstrates the hepatoprotective and genoprotective potential of plumbagin and griseofulvin NDEA-induced hepatocarcinogenesis in mice model. Both compounds significantly reversed NDEA-induced physiological deterioration, behavioral abnormalities, and hepatic DNA damage. Their therapeutic efficacy was comparable to the reference drug sorafenib, with the added benefits of a natural origin, favorable safety profile, and multifaceted mechanisms of action. These findings support the development of plumbagin and griseofulvin as promising anticancer candidates for liver malignancies, either as standalone agents or in combination with current chemotherapeutic regimens.

Given their encouraging in vivo performance, further investigation into their molecular mechanisms is essential. Transcriptomic and proteomic profiling should be employed to identify critical target genes, signaling pathways, and regulatory networks modulated by these compounds. Moreover, evaluation of oxidative stress parameters and apoptotic markers such as Bax, Bcl-2, and caspases along with angiogenic regulators like VEGF and HIF-1α, will help elucidate the cellular basis of their anticancer effects. Pharmacokinetic and bioavailability studies, particularly for griseofulvin, are also crucial to facilitate translational application and appropriate dosing strategies in clinical contexts. Additionally, long-term toxicity and chronic exposure studies should be conducted to assess potential cumulative effects, delayed toxicity, and the likelihood of resistance development upon repeated administration. These future directions will pave the way toward integrating natural therapeutics into modern oncological care with improved safety, affordability, and efficacy.

Declarations

Ethical Statements

This study was approved by the Institutional Animal Ethics Committee, Banasthali University, Rajasthan, India (Approval No.: BV/IAEC/2023/4824). All experiments followed CPCSEA guidelines.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Author Contributions

Ekta Tyagi writing-original draf, data curation, resources; Anand Prakash & Rajabrata Bhuyan Conceptualization, investigation, supervision. All authors have read and agreed to the published version of the manuscript.

Competing interest

The authors have no financial or non-financial or any other competing interest to disclose.

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