Development and Evaluation of a *bignonia undulata* Self-Emulsifying Drug Delivery System (SEDDS) with Synergistic Anticancer Properties: In Vitro and In Vivo Insights

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Abstract

Lung cancer is the second leading cause of cancer-related deaths among women, and the rising incidence has highlighted the need for more effective treatments.

This study investigates the effects of a *bignonia undulata* Suspension (WT-E) prepared from non-polar extract of *bignonia undulata* on 7,12-Dimethylbenz(a)anthracene (DMBA)-induced lung cancer.

To evaluate the antioxidant activity of the WT-E, a DPPH assay was performed at concentrations of 50µg, 100µg, 200µg, and 400µg. The cytotoxicity evaluation was performed on WT-E using the earthworm regeneration experiment revealed that suspension doses ranging from 100µg to 1000µg do not exhibit anti-mitotic activity. Acute oral toxicity testing, performed according to OECD Guideline 425 (Up and Down Procedure), indicated minimal toxicity of the HWGT suspension , supporting its potential use in cancer treatment.

Lung cancer was induced in Sprague Dawley (SD) rats via intragastric injection of DMBA at a dose of 80 mg/kg body weight. Treatment with HWGT suspension and tamoxifen significantly reduced tumor incidence, volume, and burden, likely due to their antioxidant effects. The treatment also had a notable impact on hemoglobin (Hb), red blood cell (RBC) levels, superoxide dismutase (SOD), reduced glutathione (GSH), and malondialdehyde (MDA). Histopathological analysis confirmed the effectiveness of the suspension against DMBA-induced cancer.

Overall, the HWGT suspension demonstrated significant efficacy in combating DMBA-induced lungcancer, highlighting its potential as a therapeutic agent in cancer treatment.

Keywords: Lungcancer, *bignonia undulata*, HWGT

Introduction

Lung cancer is now the most commonly diagnosed cancer in the world, with an estimated 2.26 million new cases in 2020, and it remains the leading cause of cancer mortality in women worldwide [2]. The incidence of lung cancer continues to rise globally, particularly in regions undergoing economic transformation [5]. Despite advances in its identification and treatment, which have contributed to lower mortality rates, lung cancer persists as a significant global health concern [2][5]. The incidence of lung cancer is strongly correlated with human development, and a large increase in cases is anticipated in areas of the world currently experiencing economic growth [2]. However, survival rates are much lower in less developed regions, primarily due to delays in diagnosis and lack of access to effective treatment [2]. One of the key challenges in lung cancer care is managing the heterogeneous older population, with over 30% of all patients being over 70 years old at the time of diagnosis [4]. This demographic is expected to grow in the coming decades due to the global aging population [4]. Multimorbidity in older patients is linked to increased mortality, functional impairment, poor quality of life, and higher healthcare utilization and costs, which challenge traditional healthcare systems [4]. To address this urgent global health challenge, the World Health Organization launched the Global LungCancer Initiative in 2022 [2]. This initiative aims to improve survival rates worldwide through three key pillars: health promotion, timely diagnosis, and comprehensive treatment and supportive care [2]. Future research should focus on the older patient population with relevant endpoints, both in developed and less developed countries [4]. Lungcancer represents a major global health challenge, necessitating concerted efforts to address disparities in incidence and survival rates, as well as the unique challenges posed by an aging population. Global collaboration and the implementation of initiatives like the WHO's Global LungCancer Initiative are crucial in tackling this pressing issue.

The challenges in cancer treatment, including drug resistance, severe side effects, and limited efficacy of conventional therapies, underscore the need for innovative therapeutic strategies [1]. Natural products, particularly those derived from plants, are gaining attention for their potential as anticancer agents due to their diverse biological activities and lower toxicity profiles [2]. [3]. Both are rich in bioactive compounds such as flavonoids, polyphenols, and alkaloids, which are known for their anticancer properties [4]. Studies suggest it may inhibit cancer cell proliferation and induce apoptosis in various cancer types [6 [8]. The bioactive compounds in *bignonia undulata* can modulate multiple molecular targets involved in cancer progression. These include antioxidant activity, where both plants exhibit strong antioxidant properties, mitigating oxidative stress associated with cancer development [9]; anti-inflammatory effects, where reducing inflammation helps prevent the microenvironment that supports tumor growth [10]; and induction of apoptosis, where certain phytochemicals can trigger programmed cell death in cancer cells, providing a mechanism to counteract tumor survival [11]. The exploration of BIGNONIA UNDULATAas sources of novel anticancer agents reflects a broader trend towards integrating natural products into cancer therapy [12]. Their rich content of bioactive compounds offers a promising avenue for developing effective treatments with fewer adverse effects, addressing the critical challenges faced in conventional cancer therapies [13].

Continued research into their mechanisms and clinical applications is essential for translating these findings into therapeutic strategies [14].

Natural products have long been a cornerstone in the search for therapeutic agents, with a rich history of use in traditional medicine. Plant-derived compounds, in particular, have garnered significant attention for their potential anticancer properties. The contemporary focus on these natural products involves rigorous scientific investigation to validate their efficacy and safety, aiming to translate these findings into effective clinical treatments.

Natural products have been utilized for medicinal purposes since ancient times, with many of their active components becoming available in chemically pure forms only in the past century. Despite this long history, natural products continue to play a crucial role in drug discovery. Today, approximately 80% of drug molecules are derived from natural products or their derivatives, underscoring their ongoing importance in the development of new therapeutic agents Numerous natural products have been isolated and studied for their anticancer properties, including their ability to induce cytotoxicity, inhibit cell proliferation, promote apoptosis, and suppress metastasis and angiogenesis. Plant-derived compounds, in particular, have shown significant promise in preclinical studies, with some advancing to clinical trials. This growing body of evidence highlights the potential of these compounds as effective cancer therapies

The mechanisms by which natural products exert their anticancer effects are diverse. These compounds often exhibit a wide range of bioactivities, including antimicrobial and cancer cell-targeting activities. Essential oils, for instance, have demonstrated the ability to target cancer cells specifically and enhance the efficacy of conventional chemotherapy drugs. Such findings reveal the multifaceted nature of natural products and their potential to complement existing cancer treatments (Bowman & Magalhães, 2018).

Recent research has increasingly focused on the scientific validation of natural products, particularly plant-derived compounds, to confirm their efficacy and safety. This rigorous scientific approach aims to ensure that these compounds can be developed into clinically viable treatments with minimal or no toxicity. The emphasis on scientific validation is crucial for transitioning promising compounds from preclinical studies to clinical applications (Asma, S. T., Acaroz, 2022).

Natural products have made a significant impact on contemporary anticancer drug development, with over 60% of current anticancer drugs being derived from natural sources. Additionally, many natural products are in various stages of clinical development, reflecting their ongoing potential as sources of novel anticancer agents. The continued exploration and development of these compounds are vital areas of interest in pharmaceutical research

In summary, natural products, especially those derived from plants, have demonstrated considerable promise in the realm of anticancer therapy. The shift towards rigorous scientific validation is crucial for confirming their therapeutic potential and ensuring their safety for clinical use. As research progresses, these natural compounds may offer new avenues for effective cancer treatments, reinforcing their importance in modern pharmaceutical development.

The present study seeks to investigate the anticancer activity of a specific natural compound using an in-vivo animal model. The Sprague-Dawley rat model, known for its susceptibility to carcinogen-induced tumors, has been selected for this research. Using 7,12-dimethylbenz[a]anthracene (DMBA), a potent carcinogen, the study aims to induce mammary gland tumors in these rats, providing a relevant system to evaluate the potential therapeutic effects of the compound .The primary objectives include assessing the compound's ability to inhibit tumor growth and progression by measuring parameters such as tumor incidence, size, volume, and overall burden. Additionally, the study will determine the safety profile of the compound by monitoring hematological and biochemical parameters to identify any adverse effects or toxicity associated with it (Shaik & Katari, 2022).. Understanding the mechanisms through which the compound exerts its anticancer effects is also crucial, involving an analysis of changes in molecular and cellular markers linked to tumor growth and regression (Asma & Acaroz, 2022).

This comprehensive approach aims to provide valuable insights into the therapeutic potential of the test compound. By evaluating its anticancer efficacy, safety, and mechanisms of action, the study could contribute to the development of novel anticancer therapies that enhance treatment outcomes while minimizing adverse effects

The outcomes of this research will not only add valuable information to the field of cancer therapeutics but also potentially pave the way for future clinical studies and therapeutic applications. Through detailed in-vivo evaluation, this study aspires to bridge the gap between preclinical research and clinical application, advancing the search for effective cancer treatments.

METHODS

2.1 Formation of Suspension

The suspension was made by blending components according to the given formula: 5.0% w/w soya oil as the oil phase, 84.0% w/w distilled water as the aqueous phase, 7.0% w/w Polysorbate 80 (Tween 80), and 4.0% w/w extract. The necessary amounts of extracts were weighed and added to the oil phase. The combination, which included Polysorbate 80 (Tween 80) and the extract, was heated to 75 ± 2°C. Similarly, the water phase was heated to the same temperature. The water phase was then progressively added to the oil phase, with a mechanical stirrer keeping agitation at 600 rpm for 2 hours (Seibert & Bautista-Silva, 2019).

2.2 1,1-diphenyl-2-picryl hydrazyl (DPPH) Assay

The free radical scavenging activity of the suspension was tested using a 1,1-diphenyl-2-picryl hydrazyl(DPPH) technique. A total of 4 milligrams of DPPH were dissolved in 100 mL of methanol for making the stock solution. Filtration of DPPH stock solution using methanol. In a test tube, DPPH solution (1.0 ml) was added to 1.0 ml of plant extract combination at different concentrations (25-200µg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517nm. Ascorbic acid at various concentrations (20-160µg/ml) was used as standard. The percentage of free radical inhibition was calculated as IC50.It denotes the concentration of the sample required to scavenge 50% of DPPH free radical. Ascorbic acid was

used as standard. (Baliyan & Mukherjee, 2022). The capability to scavenge the DPPH radical was calculated using the following

Formula,

% of antioxidant activity= [(Ac−As) ÷Ac] × 100

Where Ac - Absorbance of Contro

As – Absorbance of test sample

2.3 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay-

The MTT assay was carried out on MCF-7 cells to determine the cytotoxicity of the investigated compounds. The compounds were used in repeated dilutions, and cytotoxicity was determined by graphing cell viability versus concentration. The following formula was used to calculate cell viability:

Cell viability formula = (Absorbance of sample/Absorbance of positive) x100

Cells were seeded in 96-well plates at a density of 1.5 × 10^4 cells per 200 µl of media, and incubated at 37 °C for 24 hours. Following incubation, the cell medium was withdrawn and the cells were exposed to varying amounts of the compounds. Following a 24-hour incubation at 37 °C, 20 µL of MTT dye solution (5 mg/mL) in PBS was added to each well and incubated for 3 hours at 37 °C. After removing the MTT-containing medium, rinsed the plates with 100 µl of PBS. After the wash, add 200 µl of DMSO to each well and shake the plates for 5-10 minutes. The solution's absorbance was measured at 580 nm using a BMG LABTECH microplate reader.

2.4 Earthworm regeneration assay (Anti-mitotic assay)

Healthy adult earthworms of similar sizes, ranging from 1.0 to 1.5 g in body weight, were carefully selected for the experiments. Earthworms were cut with a sterile scalpel blade on the 30th segment from the mouth and the posterior segments were amputated, while the anterior parts of the body including the clitellum region were kept in separate beds and monitored every 24 h, with special attention paid to the amputation region for wound healing and/or formation and growth of the blastema. Earthworms were injected between the clitellum and the amputation region with 10 µL of distilled water or aqueous drug solution. Injections were performed at 24-h intervals for 5 or 7 consecutive days. Five worms were used for each treatment and all experiments were performed at least 3 times.

2.5 Animal studies

Experimental animals

The experiment involved young virgin female Sprague-Dawley rats weighing between 150 and 200 grames. The rats were kept in separate polypropylene cages with rice husk bedding. The experiment was conducted under typical laboratory settings, with a temperature of 25 ± 2ºC, relative humidity of 55 ± 10%, and a 12-hour light/dark cycle throughout.

Animals required

This study employed young virgin female Sprague-Dawley rats, aged 6-8 weeks and weighed between 150 and 200 grammes. The National Institute of Biosciences, Pune gave a total of 30 female rats. Each rat was kept for one to five months to ensure proper acclimatisation and monitoring.

2.5.1 Acute Toxicity study

In accordance with OECD Test Guideline 425 (Up and Down Procedure), nulliparous and non-pregnant young virgin female Sprague-Dawley (SD) rats aged 6-8 weeks and weighing between 150-200 grammes were chosen at random for the experiment. Each group had six animals that had been acclimatised for seven days prior to the start of the experimental phase. The rats were weighed and fasted for 18-20 hours before to treatment. Following the instructions, each animal was given a single oral dosage of the test drug at 48-hour intervals. The first dosage for determining the unknown toxicity of the material was 175 mg/kg body weight. Animals were observed for toxicity at intervals of ½ hour, 1 hour, 4 hours, 24 hours, and daily. If toxicity was observed at the original dosage, the dose was lowered by 3.2. If no toxicity was detected, the dosage was raised by 3.2 times the initial dose, yielding orally delivered doses of 560 mg/kg, 1792 mg/kg, and 2000 mg/kg. If no toxicity was found at these levels, a final dosage of 5000 mg/kg was given orally. All animals were observed for 14 days for indicators of toxicity, including as changes in body weight, hair, eyes, behaviour, faecal consistency, mucous membranes, convulsions, tremors, itching, coma, and death. On the 15th day, the animals were euthanised, and their brains, livers, and kidneys were removed, weighed, and kept in 10% formalin for histological investigation (OECD 2022, Test 425). One control group got distilled water orally, while the five test groups received oral dosages of 175 mg/kg, 560 mg/kg, 1792 mg/kg, 2000 mg/kg, and 5000 mg/kg, respectively. Each group contained one animal. (OECD, 2022).

2.5.2 Study procedure for *In-Vivo* anticancer study

The in-vivo anticancer study used 24 young, virgin Sprague-Dawley female rats. These rats, who had an average lifetime of three years and began reproducing at 50 to 60 days of age, were capable of having 7 to 10 pregnancies and generating 7 to 10 pups in each litter. Mammary gland tumours were produced by giving 80 mg/kg of 7,12-dimethylbenz(a)anthracene (DMBA) diluted in 1 mL of soy oil by intragastric gavage. Once the tumours grew, the treatment dose of 800 mg/kg was given three times per week for the duration of the experiment. Throughout the trial, the rats were given suitable pellet food and filtered water, and physical exams were performed on a weekly basis. Each rat has six pairs of mammary glands, which were inspected via visual examination, palpation, and touch. At the conclusion of the research, all animals were euthanised and a full autopsy was done. The mammary tumours were measured and weighed, with tumour burden, volume, yield, incidence, and mass recorded. Representative tumour samples were fixed in 10% formaldehyde, embedded in paraffin, sectioned at 5 mm intervals, stained with haematoxylin and eosin, and viewed with a light microscope.The study divided participants into four groups: Group I, the control group, received distilled water orally; Group II, 80 mg/kg of DMBA intragastrically; Group III, 80 mg/kg of DMBA intragastrically along with 6 mg/kg of Tamoxifen orally; and Group IV, 80 mg/kg of DMBA intragastrically along with the treatment dose of 800 mg/kg orally. The study included six animals per group, for a total of 24 rats

Evaluation Parameters

Parameters for Tumor Evaluation

Roy and Chakraborty (2019) used numerous metrics to evaluate tumours in Sprague-Dawley rats. Tumour incidence was calculated as the proportion of rats who developed at least one

tumour throughout the research period. The tumour volume was determined using the formula. Tumour volume = 34 x (D12)(D22)(D32).\text{Tumor Volume} = \frac{3}{4} \times \left(\frac{D1}{2}\right) (\frac{D2}{2}) - (\frac{D3}{2}Tumour volume = 43×(2D1)(2D2)(2D3), where D1D1D1, D2D2D2, and D3D3D3 are the three tumour diameters (in mm). The tumour burden was calculated by multiplying the tumour volume by the number of tumours per rat. At the end of the experiment, each tumor's diameter was measured with a Vernier calliper. Furthermore, tumour mass was measured by weighing the tumours of each animal at the conclusion of the research period, providing detailed information on tumour growth and features. (Roy and Chakraborty, 2019).

Hematological Parameters in blood sample

The effect of the test medication on hematological parameters was assessed by measuring hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC) in blood samples, as detailed by Emam et al. (2022). Blood samples were collected at three key stages throughout the trial: initially, before the induction of lungcancer; subsequently, prior to the commencement of therapy; and finally, at the end of the study **(**Emam & Abdel, 2022).

Preparation of tissue homogenenate for biochemical estimation

The animals were beheaded shortly after blood was drawn at the end of the study. The tumours were removed, dissected, cleaned in isotonic saline, and weighed. A 10% (w/v) tissue homogenate was produced with 0.1 M phosphate buffer (pH 7.4). This homogenate was then used to assess superoxide dismutase, lipid peroxidation, and reduced glutathione. (Ashraf & Kalaichelvan,2021).

Evaluation of Tumor Tissue antioxidant Biomarkers

Estimation of Superoxide Dismutase (SOD) Activity

To determine superoxide dismutase (SOD) activity, equal quantities of tissue homogenate supernatant and distilled water were combined. Then, 0.25 mL of ice-cold ethanol and 0.15 mL of ice-cold chloroform were combined. The mixture was well mixed and centrifuged at 600 g for 15 minutes at 4°C using a cyclomixer. The resultant supernatant was then combined with 0.5 mL of EDTA solution. The reaction was started by adding 0.4 mL of epinephrine, and the optical density per minute was measured at 480 nm versus a reagent blank. (Ashraf & Kalaichelvan,2021).

Estimation of Reduced Glutathione (GSH) Activity

To determine reduced glutathione (GSH) activity, equal quantities of tissue homogenate supernatant and 20% trichloroacetic acid (TCA) were mixed. The mixture was then centrifuged at 600 g for 15 minutes at 4°C before adding 2 mL of DTNB reagent to 0.25 mL of supernatant. The final volume was reduced to 3.0 mL using phosphate buffer. The produced colour was evaluated at 412 nm against a reagent blank (Henry & Ranjan , 2020), (Ashraf & Kalaichelvan,2021).

Estimation of Malondialdehyde (MDA) Activity

To determine malondialdehyde (MDA) activity, 75 µL of supernatant was mixed with 25 µL of 3N NaOH, 125 µL of H_3PO_4, and 125 µL of thiobarbituric acid. The mixture was heated at 90 °C for 45 minutes. After heating, an equal volume of butanol and pyridine (1:1) was added, and the mixture was centrifuged at 3000 g for 10 minutes using a vortex mixer. The butanol

layer (20 µL) was removed and collected, with absorbance measured at 532 nm (Ashraf & Kalaichelvan,2021).

Histopathology

Several processes were required for histopathological investigation of lungtumours. At the end of the trial, the animals were euthanised via cervical dislocation, and their tumours were removed and fixed in 10% formalin. The tumour specimens were then processed for histological analysis. After being embedded in paraffin, the tissue slices were stained with haematoxylin and eosin. The dyed slides were then inspected using a light microscope to detect pathological changes (Karnam & Ellutla, 2017).

3.0 Statistical Analysis

Results were presented as mean ± SEM. To compare the treatment groups to the positive control, one-way ANOVA followed by Tukey's test and two-way ANOVA followed by Tukey's post-test were used. Statistical analyses were carried out using GraphPad Prism 8. A p-value < 0.05 was considered statistically significant, while a p-value < 0.001 indicated a high degree of significance.

4.0 Results

4.1 *BIGNONIA UNDULATA* Suspension (WT-E)-

4.2 DPPH Assay

The antioxidant activity of the formulation was carried by 1, 1-diphenyl-2 picrylhydrazylusing a UV-visible spectrophotometer. Anti-oxidative properties of formulation were assessed in a concentration-dependent way.

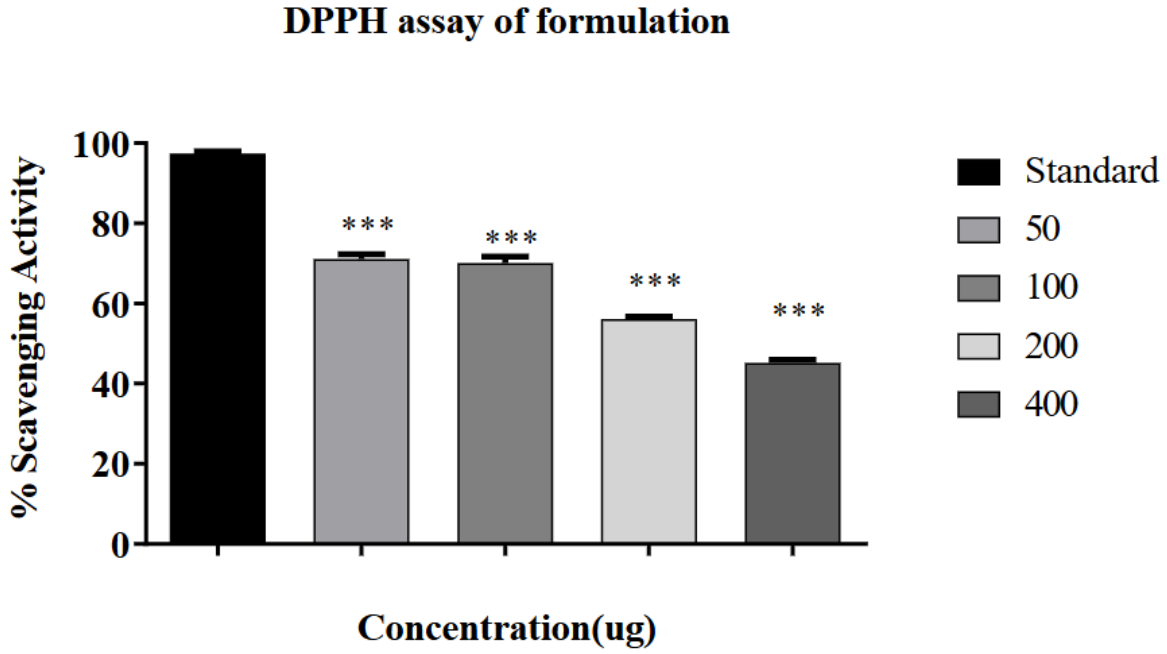

Figure 1: Free radical scavenging activity of WT-E formulation using DPPH assay.

Each value represents the mean ±SEM of 3 observations per group. Graph represents concentration (µg) V/S % Scavenging activity (One-way ANOVA followed by Tukey's post hoc test).Where significance at ***P<0.001 when compared with standard.

Figure 1 represents the graph of concentration V/S % scavenging activity of formulation which reveals antioxidant effect of Formulation performed using DPPH Assay which shows that as the concentration increases the free radical scavenging activity increases.

The findings demonstrated that the WT-E led to a significant decrease in % scavenging activity as the concentrations increases (*p≤0.05, **p ≤ 0.01, ***p ≤ 0.001) when compared to the standard group.

4.3 MTT assay

The cytotoxicity of WT-E was assessed using MTT assays at various doses (0–100 µg/ml) on preadipocytes derived mouse embryonic fibroblasts cells(3T3-L1) and MCF-7 lungcancer cells in this study. The experiments, performed in triplicate, were designed to ascertain the specific toxicity of WT-E. The data, represented as means ± standard deviation, were subjected to statistical analysis using ANOVA, followed by a post-hoc Tukey test to determine the significance levels (****P* < 0.0001) (Figure 2).

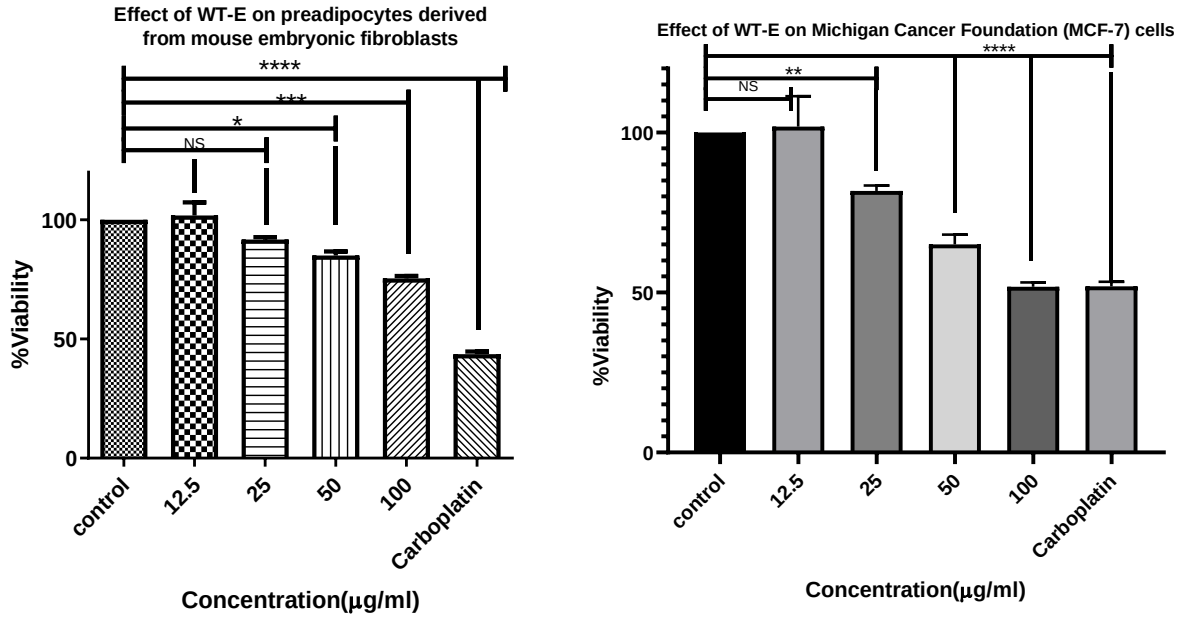

Figure 2 Cytotoxicity study of WT-E using MTT assay.

This study aimed to evaluate the cytotoxic effects of WT-E using MTT assays on 3T3-L1 normal mouse embroy fibroblast cells and MCF-7 lungcancer cells. WT-E concentrations ranging from 0 to 100 µg/ml were tested. The assay was performed in triplicate to confirm the reliability of the data. The investigation showed that cell viability in normal 3T3-L1 fibroblast

cells maintained between 75–100% for all tested WT-E dosages, suggesting less cytotoxic effect compared to the untreated control group.

Carboplatin was used as a standard for comparison, showed a considerable level of cell-killing ability compared to both the control group and different doses of WT-E. This highlights the effectiveness of Carboplatin as a reference point in our experiments.

On the other hand, there was a clear and direct relationship between the amount of WT-E and the level of cell death in MCF-7 lungcancer cells. As the concentration of WT-E increased, cytotoxicity also increased. This increasing toxicity demonstrates WT-E promise as an anticancer agent, specifically targeting lungcancer cells.

Based on the gathered data, we determined that the IC50 value of WT-E for MCF-7 cells is 45 µg/ml. This figure shows the concentration at which WT-E is capable of inhibiting 50% of cell viability, acting as a critical indicator of its cytotoxic effectiveness against cancer cells while demonstrating its protective impact on normal cells.

4.4 Antimitotic Activity (Earthworm Regeneration Assay)

Regeneration assay was performed to analyze the anti-mitotic activity of the formulation. The effect of formulation on earthworm is shown in figure 3. Formulation of concentration 100µg, 500µg and 1000µg was injected every 24 h for a period of 7 consecutive days. The regeneration of the blastema was observed after 3, 5, or 7 days in all control and treated worms. The development of blastema was observed at day 3 in control group but there is no regeneration of blastema in treatment groups. On day 5 there is regeneration in all treatment groups. The development of blastema even at higher concentration treated group shows that formulation doesn't have anti-mitotic activity. The graph (Figure 6.10) represents the concentration V/S % regeneration of blastema in each group on 5th and 7th day of treatment

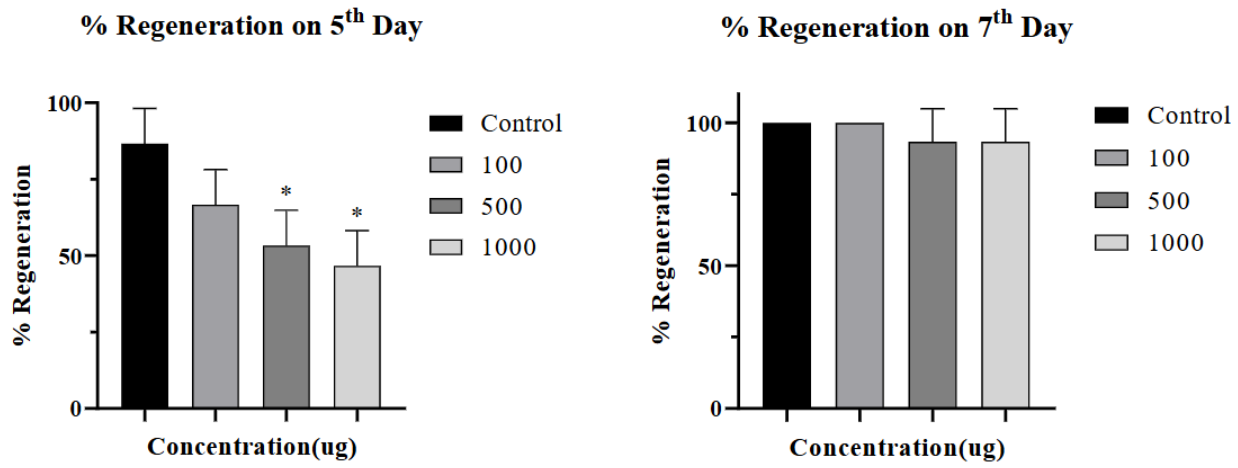

Figure 3: % Regeneration of Earthworms on Day 5 and Day 7 at different concentrations of formulation.

The graph represents concentration V/S % regeneration on 5th day and 7th day. The differences among groups were analyzed by analysis of variance (One way ANOVA) followed by Tukey's multiple comparisons post hoc test. (n=5, Where significance at *P ≤ 0.05 when compared with control. values are expressed as mean ± SEM).

Table 1- Effect of different concentration of suspension on regeneration in the Control and Treatment groups.

Day	Control	100 µg	500 µg	1000 µg
5th	86.67± 6.66	66.67± 6.66	53.33± 6.66	46.67± 6.66
7th	100± 0	100± 0	93.33± 6.66	93.33± 6.66

The differences among groups were analyzed by analysis of variance (One way ANOVA) followed by Tukey's multiple comparisons post hoc test. (n=5, Where significance at *P ≤ 0.05 when compared with control. values are expressed as mean ± SEM**)**

Acute Oral Toxicity

Acute toxicity of the formulation was assessed using Sprague Dawley female rats in accordance with OECD guidelines 425.All animals were continuously observed for behavioural changes and other sign of toxicity or mortality up to a period of 14 days. The body weight, food intake and water intake were also observed on 1st, 7th and 14th day (OECD, 425). There were no any physical and behavioural changes observed in any animal during observation period. It was noted that normal food and water consumption along with minimal changes in body weight were occurring. Body weight of all animals did not reveal any significant change. Mortality was not found in any group.

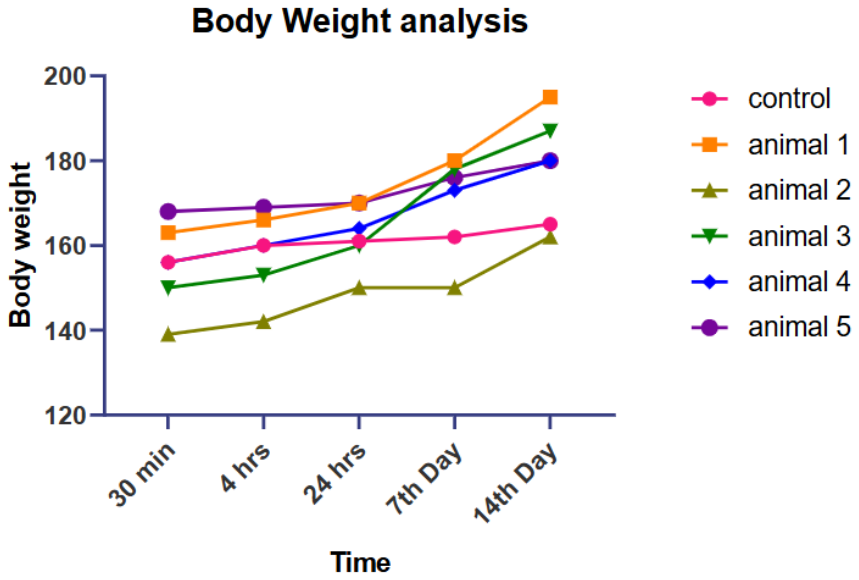

Figure 4 - Change in body weight during the experimental period of 14 days in control and experimental animals.

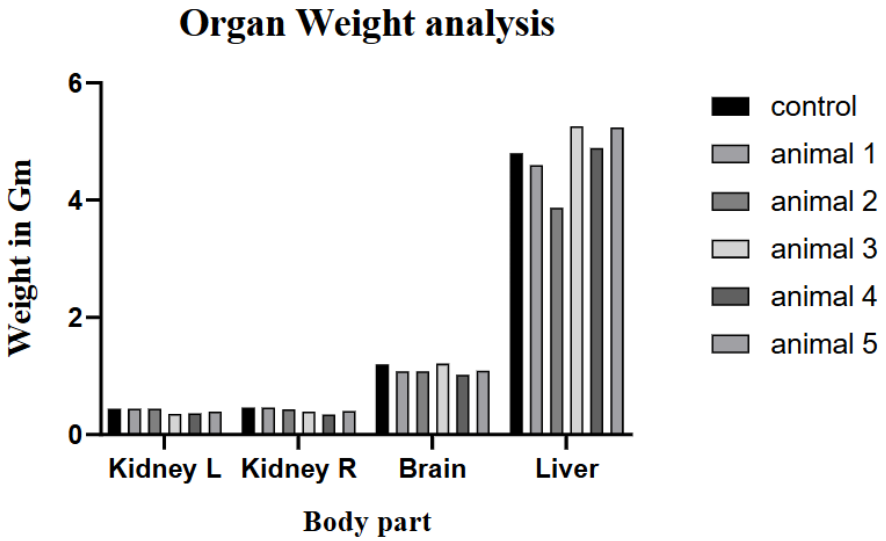

Figure 5- organ weight analysis of experimental animals of acute oral toxicity

Finding organ weight is one of the most crucial components of toxicological research. Numerous widely accepted guidelines recommended weighing vital organs such the liver, kidney, heart, brain, and others (Abebe, 2023).

Histopathology:

Figure 6 gives the idea about histological changes in control and experimental animals. The images of liver are taken at 10 x and 40 x for the assessment of histological changes. The images of liver of control rat are showing normal architecture of rat liver. The images of liver of Animal 1, Animal 2 and Animal 3 are showing normal architecture of rat liver and no patchy necrosis was observed. These are the animals treated at dose 175 mg/kg and 560 mg/kg and 1792 mg/kg of formulation. The images of liver of Animal 4 and Animal 5 rat are showing patchy necrosis in rat liver. From the result and observation we can say that the animals treated at dose, 2000 mg/kg and 5000mg/kg are having histological toxicity at some extend.

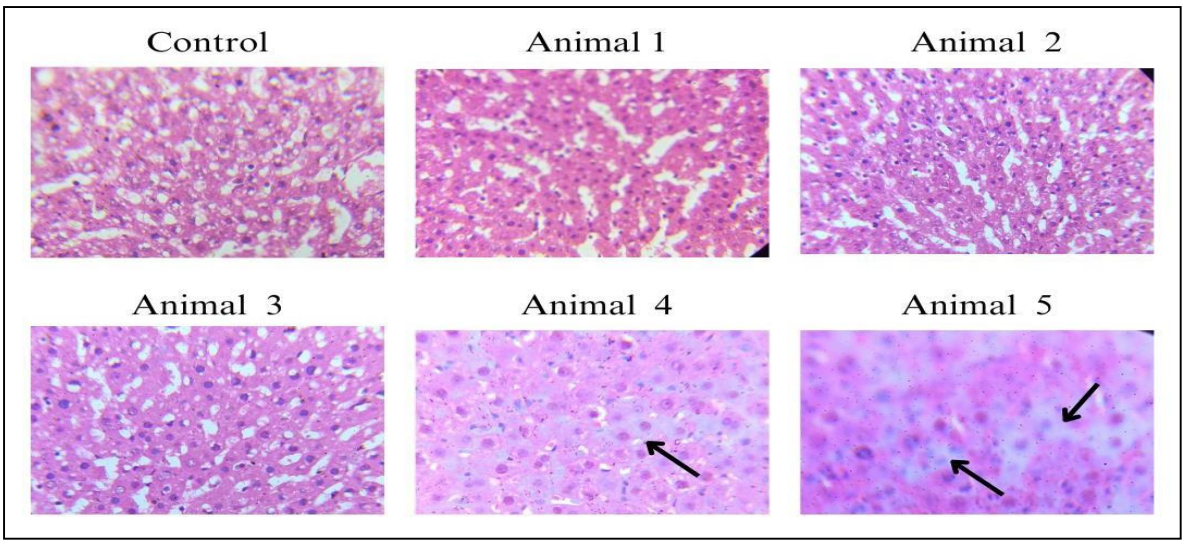

Figure 6 - Histopathological analysis of acute oral toxicity study

In-vivo anti-cancer study

Mammary tumour induction by DMBA is one of the most used techniques among the numerous animal models for researching the various features of lungcancer. The goal of the current study was to assess anti-lungcancer efficacy of HWGT suspension in DMBA-induced rats.

Effect on body weight

Body weight is one of major parameter in any disease condition. Change in body weight during the study time is shown in figure 7. In control group there is increase in body weight is observed. After giving DMBA to Disease control group, Standard group and formulation treated group it was observed that there is decrease in body weight of rats and it decreases till end of the study in disease control group.

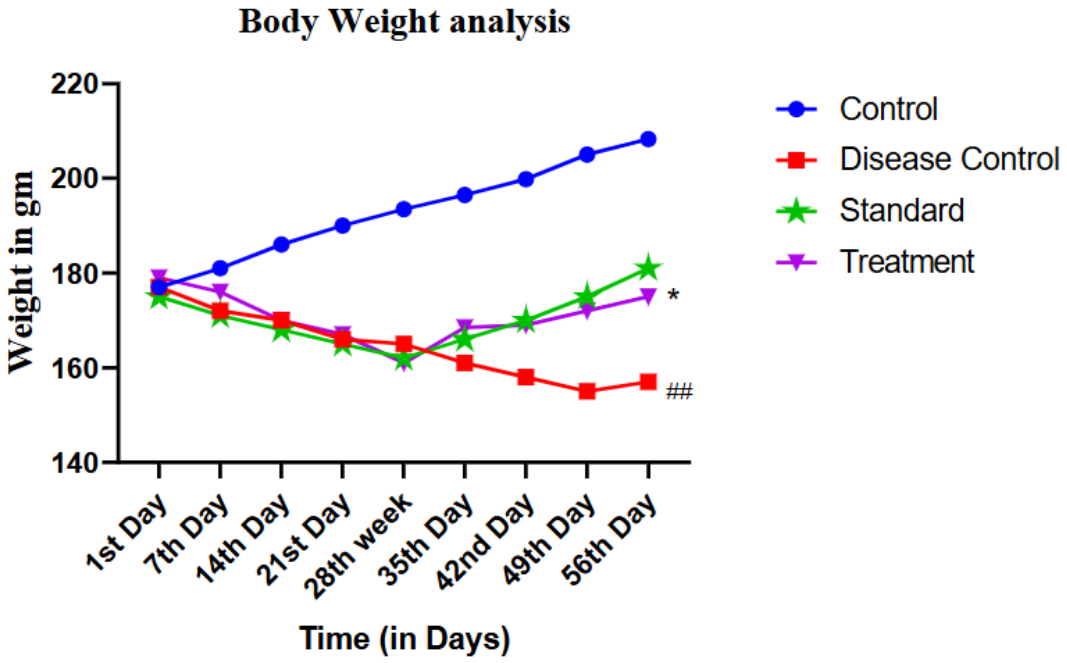

Figure 7 - Body weight analysis of experimental animals

Graph represents Time V/S Body Weight. The differences among groups were analyzed by analysis of variance (One way ANOVA) followed by Tukey's multiple comparisons post hoc test. (n=6, Where significance at $^{\#\#}P<0.001$ when Disease group is compared with control group and $*P \leq 0.05$ when treatment group compared with Disease group. values are expressed as mean ± SEM)

Effect of formulation on tumor parameter

Table 2 and Figure 8 confirms the effect of HWGT suspension on DMBA induced tumors. Tumor size is high in DMBA control group, whereas in standard and suspension treated groups, the decrease in tumor size was evident.

Table 2: Tumor Parameter Analysis

	Control	Disease control	Standard	Treatment
Tumor volume	0	3.867±0.2480$^{\#\#\#}$	1.875±0.6194*	2.150± 0.4674*
ıor weight (gm)	0	4.058±03120$^{\#\#\#}$	1.692±0.5618**	2.175± 0.4360*
Tumor burden	0	3.867±0.2480$^{\#\#\#}$	1.875±0.6194*	2.150± 0.4674*
Tumor incidence (T/rat)	0	100%	66.70%	83.30%

Table 2 shows the effect of suspension on tumor characteristics The differences in tumor among groups were analyzed by analysis of variance (One way ANOVA) followed by Tukey's multiple comparisons post hoc test. (n=6 Where significance at $^{\#\#\#}P \leq 0.001$ when Disease group is compared with control group and $^{*}P \leq 0.05$, $^{**}P \leq 0.01$ when treatment group compared with Disease group. values are expressed as mean ± SEM)

Tumor incidence was 100% in DMBA control group (***$p < 0.001$) while, decrease in the tumor incidence was observed in standard and suspension treated group. This demonstrates that tumour growth was successfully induced in the disease control rat. Similarly, tumor weight (*$p \leq 0.05$), and tumor volume (*$p \leq 0.05$) were decreased with treatment compared to DMBA control group. Comparing the disease control group to the control group, there was a statistically significant rise in tumour volume, tumour weight, and tumour burden ($^{\#\#\#}P \leq 0.001$).

Discussion

Lungcancer is the most common cancer among women and the leading cause of cancer-related deaths in over 100 countries (Bray et al., 2018). Due to the high mortality and severe side effects of conventional treatments, many patients seek alternative therapies. Plant-based natural products, known for their chemotherapeutic properties, show promise in treating and preventing cancer with fewer side effects (Moga et al., 2016; Yuan et al., 2016). Herbal therapies, combined with chemotherapy, are effective, cost-efficient, and reduce toxicity and drug resistance (Nobili et al., 2009; Ijaz et al., 2018; Mangla et al., 2018). Wang et al. demonstrated the synergistic chemopreventive effect of berberine and curcumin on lungcancer. Building on this, the present study evaluated the synergistic effect of non-polar extracts from *bignonia undulata*

This study assessed the chemotherapeutic effectiveness of an HWGT formulation against mammary gland cancer in young female Sprague-Dawley rats exposed to DMBA. Both the HWGT formulation and Tamoxifen showed inhibitory effects on DMBA-induced mammary cancer.

Suspension s, including nanosuspension s stabilized by emulsifiers, enhance cancer therapy by improving drug delivery, bioavailability, and controlled release in immiscible liquids (Utreja et al., 2010). They are used to deliver anticancer drugs with poor aqueous solubility, increasing drug stability and uptake by cancer cells (Miranda et al., 2021). Suspension s can also enable targeted drug delivery by modifying droplets with ligands, such as antibodies, for specific binding to cancer cells, enhancing drug concentration at the tumor and reducing systemic toxicity (Mahato, 2017; Sánchez et al., 2019).

Antioxidants protect cells from oxidative damage caused by Reactive Oxygen Species (ROS), which are linked to various diseases. They enhance the body's antioxidant defenses, reduce ROS production, and inhibit free radical formation (Srinivasan et al., 2022; Supasuteekul et al., 2016).

The antioxidant activity of the HWGT formulation was assessed using the DPPH assay at concentrations of 50, 100, 200, and 400 µg. Ascorbic acid, the positive control, showed strong radical scavenging activity. The HWGT formulation demonstrated 45% scavenging activity at 400 µg and 56% at 200 µg. The DPPH assay measures radical scavenging by detecting color changes, indicating the reduction in DPPH radicals (Kedare et al., 2011). Results showed that increased concentration led to decreased scavenging activity.

The IC50 value derived from the assay was instrumental in confirming the dosage of the HWGT formulation administered in animal treatment.

Antimitotic activity is essential in cancer treatment, targeting cell division and growth. Plants offer valuable antimitotic agents, with tubulin and microtubules being key targets for drugs like taxanes and vinca alkaloids (Dall'Acqua, 2014). Studies link antimitotic activity with effective cancer treatment (Molina et al., 2021; Patil et al., 2004).

Eisenia eugeniae, an earthworm model known for its rapid mitosis and regeneration, was used to assess the antimitotic potential formulation. The study found no cytotoxicity, with blastema regeneration occurring by the fifth day, indicating that the HWGT formulation does not exhibit antimitotic activity at concentrations from 100 µg to 1000 µg.

While formulation have known pharmacological benefits, their potential toxicity has been less studied. Acute toxicity studies of individual extracts have been conducted (Das et al., 2014; Shrivastava et al., 2022). This research assessed the acute toxicity of the HWGT suspension , which is crucial for determining a safe dose range (Khanna et al., 2016).

No mortality was observed over a 14-day period. Body and organ weights showed no significant changes at doses from 175 mg/kg to 5000 mg/kg, suggesting low toxicity. Behavioral assessments showed no adverse effects. However, histopathological examination revealed patchy necrosis in liver tissues at doses of 2000 mg/kg and 5000 mg/kg, indicating potential liver toxicity at higher doses. Overall, the formulation appears generally non-toxic in rats.

Carcinogenesis induced by DMBA involves the breakdown and disruption of tissue redox equilibrium, leading to oxidative stress, biochemical alterations, and pathophysiological changes in animal studies (Krishnamoorthy and Sankaran, 2016). Sprague-Dawley rats are typically used in DMBA-induced carcinogenesis studies to develop lungtumors. A single dose of DMBA has been shown to be a potent inducer of lungcancer (Alvarado et al., 2017; Alvarado et al., 2016; Lopes et al., 2014).

Reactive oxygen species (ROS) exacerbate membrane lipid peroxidation, disrupting

regular metabolic functions and causing body weight loss, which promotes the onset and progression of cancer (Saha et al., 2017). A previous report indicated that the decreased body weight among STZ-induced diabetic rats was reversed upon administration of Ocimum sanctum leaf powder, likely due to its antilipidemic properties (Suanarunsawat et al., 2016).

Our study confirms that the HWGT formulation, with *bignonia undulata*, exhibits potential chemopreventive effects against DMBA-induced mammary gland cancer. It not only inhibits tumor growth but also reduces oxidative stress and biochemical changes linked to carcinogenesis.

The acute oral toxicity study showed no significant toxicity at tested doses, though higher doses caused patchy liver necrosis, highlighting the need for dose optimization. Suspension s as a delivery system for the bioactive compounds in BIGNONIA UNDULATAenhance solubility, stability, and targeted delivery, improving therapeutic outcomes and minimizing systemic toxicity, making them a promising strategy for safer and more effective cancer treatments.

In conclusion, the HWGT formulation shows strong potential as a chemopreventive agent against lungcancer with minimal toxicity at most doses. The suspension -based delivery system enhances its therapeutic effectiveness. However, additional research, including chronic toxicity studies and clinical trials, is needed to fully assess its safety and efficacy. These findings support the use of natural products in cancer therapy and emphasize the need for further development of safe and effective herbal formulations..

DMBA (80 mg/kg) reduced body weight in rats during induction, with the disease control group showing significantly lower body weight compared to the control group (##P≤0.001). Treatment with tamoxifen (6.6 mg/kg) and the HWGT formulation (800 mg/kg) led to weight gain, with both treatments showing increased body weight compared to the disease group.

The formulation treatment group exhibited reduced tumor volume, weight, and burden compared to the disease control group. Tamoxifen showed slightly greater reductions in tumor metrics than the formulation. Both treatments effectively inhibited cancer growth.

Haematological tests revealed that DMBA significantly decreased RBCs and hemoglobin levels in the disease control group, indicating potential anemia, a common side effect of cancer (Shrivastava et al., 2017; Gaspar et al., 2015). Cancer-induced anemia often results from iron deficiency and impaired red blood cell formation (Zingue et al., 2018).

The study found that HWGT formulation improved blood parameters, with Hb, RBC, and platelets decreasing after DMBA administration, indicating cancer-related inflammation. Treatment with HWGT and tamoxifen significantly normalized these parameters compared to the disease control group.

HWGT formulation demonstrated potential antioxidant activity, increasing SOD and GSH levels, and reducing MDA levels, which contributed to reduced tumor size and incidence. Antioxidants prevented oxidative damage and helped control cancer progression (Sharma et al., 2020; Latif et al., 2021; Uddin et al., 2020; Pizzino et al., 2017).

Histopathology revealed severe changes in mammary tissue due to DMBA, including hyperplasia and neoplastic cells. Tamoxifen and HWGT suspension improved cellular architecture, with HWGT showing moderate improvement compared to tamoxifen (Karnam et

al., 2017; Kunnumakkara et al., 2019; Mundhe et al., 2015; Nassan et al., 2018; Zingue et al., 2018).

In summary, HWGT suspension demonstrated significant anti-cancer activity, comparable to tamoxifen, suggesting its potential as an alternative or complementary therapy for lungcancer (Emam et al., 2022; Gibson et al., 2013).

Conclusion

This study demonstrated that the HWGT *bignonia undulata* suspension effectively inhibited DMBA-induced mammary cancer in Sprague-Dawley rats, showing similar efficacy to the standard drug Tamoxifen. The formulation exhibited minimal acute toxicity, with only slight liver toxicity observed at higher doses. The results suggest that HWGT could serve as a promising natural alternative in cancer therapy, offering potential benefits in enhancing treatment efficacy and reducing adverse effects. Further research is recommended to optimize dosage and evaluate long-term safety in clinical settings.

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