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Toxicological Evaluation and Cytotoxic Potential of *Eclipta Prostrata* Leaf Extract in Experimental Models

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Abstract

This study aimed to evaluate the toxicological safety and cytotoxic potential of the ethanolic extract of Eclipta prostrata leaves (EEEP) through *in vivo* and *in vitro* experimental models. The investigation included acute and chronic oral toxicity tests in mice, a brine shrimp lethality bioassay, and an in-vitro cytotoxicity assay using HeLa cervical cancer cells. Acute toxicity was assessed at doses up to 3000 mg/kg, while chronic toxicity involved daily administration of 500 mg/kg for 150 days in Swiss-albino mice. Biochemical, hematological, and antioxidant parameters were analyzed post-treatment. Cytotoxic activity was determined using the brine shrimp lethality assay, and MTT-based assays. No mortality or toxicity symptoms were observed in either acute or chronic studies. Physiological, biochemical, and antioxidant profiles remained within normal ranges. The brine shrimp assay showed strong cytotoxic potential with LC_{50} = 39.273 µg/ml, while in-vitro testing indicated moderate cytotoxicity against HeLa cells (IC_{50} > 1024 µg/ml). EEEP is non-toxic under experimental conditions and exhibits notable cytotoxic potential, suggesting the presence of bioactive compounds warranting further chemopreventive and anticancer investigation.

Keywords: Eclipta Prostrata, Ethanolic Extract, Toxicological Evaluation, Brine Shrimp Lethality Assay, Cytotoxicity, HeLa Cell Line, Anticancer Activity, Chemopreventive Potential

1. Introduction

For centuries, plants have served as one of the most vital resources for the treatment and prevention of various diseases. Medicinal plants play a significant role in the healthcare system, particularly in developing nations where access to modern medicines may be limited. The World Health Organization (WHO) estimates that over 80% of the global population depends on traditional medicines, the majority of which are derived from plant sources. These plants provide bioactive compounds with therapeutic potentials such as alkaloids, flavonoids, tannins, and terpenoids [1].

In the modern pharmaceutical industry, many compounds are derived directly or indirectly from medicinal plants. These plant-based bioactive agents have contributed significantly to drug discovery, especially in the development of treatments for cancer, infectious diseases, inflammation, and skin disorders [2].

Among these, Eclipta prostrata (L.) L., commonly known as false daisy or bhringraj, has garnered increasing scientific attention due to its ethnomedicinal relevance and wide array of pharmacological effects. Traditionally used in Ayurvedic and Chinese medicine, Eclipta prostrata is often applied in liver-related disorders, skin ailments, and hair loss treatments [3, 4]. Recent studies have revealed its hepatoprotective, anti-inflammatory, antioxidant, antimicrobial, and anticancer activities [4, 5, 6].

Given the increasing burden of chronic diseases, including skin cancers, there is a pressing need to explore plant-based therapies that are effective, accessible, and exhibit fewer side effects. The investigation of Eclipta prostrata in this context may offer promising leads for the development of novel therapeutic agents targeting various pathological conditions including skin carcinogenesis [7].

Eclipta prostrata, a member of the Asteraceae family, is a fast-spreading, small, annual or perennial herb known for its sprawling growth habit and medicinal importance (See Fig 1**)**. It typically grows low to the ground, reaching a height of 20–60 centimeters, and is easily identified by its dark green, lance-shaped leaves, hairy stems, and small, solitary white flower heads. The plant often forms dense mats over moist soil and is commonly found in wetlands, rice fields, marshes, roadsides, and other damp environments.

Fig 1: Eclipta Prostrata Plant

In Bangladesh, Eclipta prostrata is widely distributed across all regions, including lowland plains, the Chittagong Hill Tracts, and rural agricultural areas. Beyond Bangladesh, the species is naturally found throughout tropical and subtropical regions of Asia, Africa, North and South America, and Australia. It holds significant value in traditional medicinal systems such as Ayurveda, Unani, and Traditional Chinese Medicine, particularly for its hepatoprotective, anti-inflammatory, and hair growth-promoting properties.

1.1 Botanical Features

Botanical data of Eclipta prostrata have been compiled from various authoritative sources.

Common Names: False daisy, Bhringraj, Kesharaj
Botanical Name: Eclipta prostrata (L.) L.
Synonyms: Eclipta alba, Verbesina prostrata
Family: Asteraceae
Used part: Leaves (commonly), whole plant

1.2 Scientific Classification (NCBI Taxonomy)

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Asterales
Family: Asteraceae
Genus: Eclipta
Species: Eclipta prostrata

1.3 Morphology

Eclipta prostrata is a small, branched, annual herb, usually found growing prostrate or ascending. The plant is characterized by its dark green, ovate leaves arranged oppositely, and small white solitary flowers. The stems are cylindrical and hairy, and the plant emits a distinct aroma when crushed. Its characteristic white flowers help in easy identification.

1.4 Distribution & Habitat

Eclipta prostrata is native to the warm temperate and tropical regions of the world. It thrives in moist, marshy places and is found throughout Asia, Africa, the Americas, and Australia. In Bangladesh, India, and other South Asian countries, it grows abundantly in paddy fields, along roadsides, and in damp wastelands.

1.5 Traditional Medicinal Uses

Traditionally, *Eclipta prostrata* has been widely used in Ayurveda, Siddha, and Unani medicine. It is known for its benefits in treating liver disorders such as jaundice and hepatitis, skin diseases, wounds, and hair loss. It is also used as a tonic for promoting hair growth, improving eyesight, enhancing memory, and managing respiratory ailments. The juice of the leaves is often applied topically for skin infections and inflammation, and used internally for its detoxifying and hepatoprotective properties.

2. Material and Methods

Plants have long been recognized as valuable sources of bioactive compounds used in traditional and modern medicine. The extraction and isolation of phytochemicals from medicinal plants have played a crucial role in the discovery of many therapeutic agents [8, 9]. For instance, compounds like quinine, morphine, and artemisinin originated from botanical sources [10]. Eclipta prostrata, commonly known as False Daisy, is traditionally used in Ayurvedic and folk medicine for treating liver disorders, skin diseases, and promoting hair growth due to its rich phytochemical profile.

Extraction methods are typically divided into two categories: [11]

- Cold extraction
- Hot extraction

Once the extraction is completed, the crude extract is concentrated and the active constituents can be further separated using methods such as chromatography. To prevent degradation or loss of material, concentrated extracts should be stored in airtight containers at low temperatures.

2.1 Study Design

2.1.1 Materials

Fresh leaves of Eclipta prostrata were used as the raw material for extraction.

2.1.2 Plant Collection & Identification

The leaves of Eclipta prostrata were collected from rural areas of Rajshahi, Bangladesh, in September 2023. The collected specimen was identified and authenticated by a taxonomist at the Bangladesh National Herbarium, Mirpur, Dhaka-1216, and a voucher specimen was submitted for

reference.
Accession no.: DACB 87056 for Eclipta prostrata

2.2 Preparation of Crude Extract
2.2.1 Drying & Grinding
Fresh leaves were thoroughly washed with clean water to remove dirt and impurities. The cleaned leaves were then shade-dried at room temperature to protect the phytochemicals from heat degradation. Once dried, the leaves were ground into a coarse powder using a mechanical grinder. The powdered material was stored in an airtight container in a cool, dry, and dark environment until further use.
2.2.2 Cold Extraction
A total of 700 gm of Eclipta prostrata leaf powder was divided equally into two glass containers (each containing 350 gm). Each portion was soaked in 1.5 L of 96% ethanol. The containers were sealed and kept for 14 days, with occasional shaking and stirring to facilitate maximum extraction of bioactive constituents.
After 14 days, the mixture was first filtered using a clean cotton cloth, followed by filtration through cotton plugs and Whatman No. 1 filter paper.
To ensure complete extraction, the remaining plant residue was re-soaked in another 1.5 L of 96% ethanol for 5 days, following the same procedure. After this period, the extract was again filtered through the same steps.
2.2.3 Evaporation of the Solvent
The combined filtrates were subjected to rotary evaporation at controlled temperature to remove the ethanol and obtain a concentrated extract. The semi-solid extract was transferred to a beaker and covered with perforated aluminum foil. It was then left under a table fan in a well-ventilated area to allow further evaporation of residual solvent.
Finally, a vacuum desiccator was used to remove any trace solvent, leaving behind a dark green viscous paste. This was labeled as crude ethanolic extract of Eclipta prostrata (EEEP) Extraction at a Glance.

2.3 Yield Determination
After the 1st extraction, 20.3 gm of crude extract was obtained from 700 gm of leaf powder:

$$\text{Yield (\%)} = (20.3 / 700) \times 100 = 2.9\%$$

After the 2nd extraction, 12.5 gm of extract was obtained:

$$\text{Yield (\%)} = (12.5 / 700) \times 100 = 1.79\%$$

$$\text{Total yield (\%)} = (2.9 + 1.79)\% = 4.69\%$$

3. Toxicological Investigation
Medicinal plants have long been used for their therapeutic potential due to their accessibility and low cost. However, despite their widespread traditional use, many medicinal plants lack comprehensive toxicological evaluations. According to the World Health Organization (WHO), the historical and traditional use of herbal medicines is often mistakenly considered as proof of safety, even though adverse effects can and do occur, especially with long-term or high-dose exposure [1].
To ensure the safety of medicinal plant extracts intended for therapeutic use, both acute and chronic toxicity studies are essential [12, 13]. Acute toxicity evaluates the immediate or short-term toxic effects within 14 days of a single or short-term exposure, while chronic toxicity assesses long-term exposure effects, typically over a period longer than 90 days. These studies are crucial for identifying potential target organ toxicity, behavioral changes, biochemical alterations, and mortality. They also help determine the No Observed Adverse Effect Level (NOAEL), which is essential for establishing human safety margins.

3.1 Acute Toxicity Test
3.1.1 Test Material
Ethanolic extract of Eclipta prostrata leaves (EEEP).
3.1.2 Experimental Animal
Young Swiss-albino mice (4–5 weeks old, 18–24 g) were used. Animals were obtained from the Animal House of Khulna University and acclimatized for one week in standard laboratory conditions (natural light cycle, temperature 22–25°C, humidity 55–65%). They were provided with standard laboratory food and water ad libitum. All procedures followed institutional ethical guidelines (Ref. No.: KUAEC-2024/03/02).
3.1.3 Experimental Design
The test was conducted according to OECD Guidelines with slight modifications [14]. Thirty-six mice were randomly divided into six groups (n=6 each). Five groups (Group I–V) received EEEP at escalating doses of 250, 500, 1000, 2000, and 3000 mg/kg body weight, respectively. Group VI served as control and received 2% Tween-80 in distilled water.
3.1.4 Preparation of Sample Suspension
The required amounts of extract (34.3, 68.6, 137.5, 274.5, and 411.75 mg for respective doses) were triturated with a small quantity of Tween-80, followed by gradual addition of distilled water to a final volume of 8 ml. Each suspension was vortexed to ensure uniformity. Mice received 1 ml of the prepared suspension orally.
3.1.5 Observation
All animals were observed continuously for 24 hours and then for 14 days for signs of behavioral changes, toxicity, or mortality (**Insert table 3.1 and 3.2 here**).

Table 3.1: Results

Group	Dose (mg/kg)	No. of treated mice	No. of dead mice after 24 hours	Mortality rate
I	250	6	0	0
II	500	6	0	0
III	1000	6	0	0
IV	2000	6	0	0
V	3000	6	0	0
VI	Control	6	0	0

Table 3.2: Body Weight Variation

Group	Day 1 (g)	Day 14 (g)
I	19.8 ± 0.65	27.3 ± 0.82
II	21.4 ± 0.51	28.5 ± 0.77
III	20.6 ± 0.72	29.1 ± 0.84
IV	22.3 ± 0.68	30.3 ± 0.71
V	21.9 ± 0.63	30.8 ± 0.69
VI	22.1 ± 0.58	**3.2** ± 0.76

3.2 Chronic Toxicity Test
3.2.1 Test Material
Ethanolic extract of Eclipta prostrata leaves (EEEP).
3.2.2 Experimental Animal
Swiss-albino mice (5–6 weeks old, 20–25 g) were

acclimatized and maintained as described earlier.

3.2.3 Experimental Design

Ten mice were randomly divided into two groups:

- **Control Group** received 2% Tween-80 in water
- **Test Group** received 500 mg/kg/day of EEEP orally for 150 days

3.2.4 Preparation of Test Sample

500 mg of EEEP was triturated with Tween-80 and made up to 5 ml with distilled water. Each mouse in the test group received a volume equivalent to 0.01 × body weight in grams.

3.2.5 Observation

Animals were monitored daily for signs of toxicity or mortality. After 150 days, animals were sacrificed for biochemical, hematological, and antioxidant assays (**Insert table 3.3, 3.4, 3.5, 3.6 and 3.7 here)**.

Results:

Table 3.3: Body Weight

Time	Control	EEEP (500 mg/kg)
Day 1	27.2 ± 0.58	23.2 ± 0.86
Day 30	28.2 ± 0.58	24.8 ± 0.80
Day 60	29.0 ± 0.94	26.8 ± 0.86
Day 90	31.0 ± 0.89	29.0 ± 0.71
Day 120	31.4 ± 0.81	30.8 ± 0.80
Day 150	33.0 ± 1.14	32.6 ± 1.03

Table 3.4: Liver and Kidney Weight (g)

Organ	Control	EEEP
Liver	1.566 ± 0.02	1.552 ± 0.03
Kidney	0.216 ± 0.0084	0.214 ± 0.004

Table 3.5: Antioxidant Enzymes in Liver Tissue

Parameter	Control	EEEP (500 mg/kg)
SOD (U/mg protein)	51.00 ± 3.22	57.52 ± 4.02
GSH (µg/mg protein)	1033.11 ± 28.42	1027.48 ± 85.49
CAT (U/mg protein)	172.68 ± 10.53	194.27 ± 1.50

Table 3.6: WBC Count (cells/µL)

Type	Control	EEEP
Total WBC	6780 ± 656.37	6600 ± 248.01
Neutrophil	2526 ± 190.07	2434 ± 71.32
Lymphocyte	3552 ± 414.14	3506 ± 155.04
Eosinophil	364 ± 42.97	356 ± 39.82
Monocyte	210 ± 11.83	194 ± 17.49
Basophil	114 ± 23.79	110 ± 18.71

Table 3.7: Biochemical Parameters

Parameter	Control	EEEP (500 mg/kg)
SGPT (U/L)	35.07 ± 2.25	31.8 ± 1.70
SGOT (U/L)	31.27 ± 5.75	27.3 ± 1.94
Urea (mg/dL)	17.93 ± 3.22	13.8 ± 1.28
Bilirubin (mg/dL)	1.03 ± 0.18	1.03 ± 0.18
Creatinine (mg/dL)	1.02 ± 0.10	1.00 ± 0.05
Total Cholesterol (mg/dL)	185.6 ± 4.11	169.73 ± 4.50
HDL (mg/dL)	81.73 ± 12.86	74.47 ± 2.05
LDL (mg/dL)	103.87 ± 9.03	93.93 ± 3.53
Triglycerides (mg/dL)	110.2 ± 12.11	97.0 ± 11.59

3.3 Discussion

The **acute toxicity** study revealed that *Eclipta Prostrata* leaf extract did not induce mortality or any observable signs of toxicity up to 3000 mg/kg dose, suggesting it is **non-toxic in acute exposure.**

In the **chronic toxicity** study, no abnormal behavior, physical abnormalities, or mortality was observed over the 150-day treatment with 500 mg/kg/day. Body weight, liver, and kidney weights showed normal progression [16]. Antioxidant biomarkers (SOD, GSH, CAT) were either maintained or slightly improved, indicating no oxidative stress. Biochemical parameters remained within normal physiological ranges, and no significant differences in WBC counts were found, indicating **absence of systemic or organ-specific toxicity** [17, 18].

4. Brine Shrimp Lethality Bioassay of Eclipta Prostrata Leaf Extract

The brine shrimp lethality bioassay is a well-established, rapid, and inexpensive method for the preliminary screening of bioactive compounds, particularly those with potential cytotoxicity [19]. This technique uses Artemia salina nauplii (brine shrimp larvae) to assess the biological activity of natural product extracts, fractions, or pure compounds. A positive correlation between brine shrimp lethality and cytotoxicity has made this assay a reliable indicator of pharmacological potential, especially for anticancer agents. In this study, the ethanolic extract of Eclipta prostrata leaves was subjected to the brine shrimp lethality bioassay to evaluate its cytotoxic potential [20].

4.1 Objective

The objective of the present investigation was to evaluate the cytotoxic activity of the ethanol extract of Eclipta prostrata leaves using the brine shrimp lethality bioassay.

4.2 Study of Cytotoxic Activity by Brine Shrimp Lethality Bioassay

4.2.1 Materials

- Artemia salina (brine shrimp eggs)
- Sea salt (NaCl + table salt)
- DMSO (Dimethyl sulfoxide)
- Micropipettes, pipettes, volumetric flasks, test tubes
- Hatchery setup: plastic bottle, air stone, pump, light source
- Beakers, petri dishes, conical flask, magnifying glass

4.3 Methodology

4.3.1 Preparation of Sea Water

A mixture of 20g pure NaCl and 18g table salt was dissolved in 1 liter of distilled water and filtered to obtain clear artificial sea water.

4.3.2 Construction of Hatchery and Hatching of Brine Shrimp

A conical bottle setup with aeration and light was used to hatch Artemia salina eggs (0.5g per 1L sea water) over 24 hours. The hatched nauplii were attracted to light and collected for the bioassay.

4.3.3 Preparation of Stock Solution

16 mg of dried ethanolic extract of E. prostrata leaves was dissolved in 25 ml sea water using a few drops of DMSO. This stock solution had a concentration of 640 µg/ml.

4.3.4 Preparation of Standard Solution

0.25 mg of vincristine sulfate (standard drug) was dissolved in sea water with DMSO and diluted to 25 ml to achieve a concentration of 10 µg/ml.

4.3.5 Preparation of Control
A control solution was prepared using only DMSO and sea water without any extract or standard.

4.4 Application of Test Solutions and Brine Shrimp Nauplii
- A total of 31 test tubes were used:
  - 14 tubes for E. prostrata extract (concentrations: 5, 10, 20, 40, 80, 160, 320 µg/ml)
  - 14 tubes for standard drug (vincristine sulfate)
  - tubes for negative control
- Each tube was filled with 2.5 ml of test solution, 20 brine shrimp nauplii, and made up to 5 ml with sea water.
- After 24 hours, surviving nauplii were counted.

4.5 Counting of Nauplii and Data Presentation
% Mortality was calculated using the following formula(**Insert table 4.5 here**):

% Mortality = [(Avg. no. of alive shrimp in control – Avg. no. of alive in test) / Avg. no. of alive in control] × 100

The test was performed in duplicate to ensure accuracy.

Table 4.5: Result of Brine Shrimp Lethality Bioassay using *Eclipta prostrata* Leaf Extract

Conc. (µg/ml)	Log. Conc.	No. of nauplii taken	Dead (Test-I)	Dead (Test-II)	Avg. Dead	Avg. Alive	Control Alive	% Mortality	LC_{50} (µg/ml)
5	0.698	20	3	2	2.5	17.5	17	2.94	
10	1.000	20	5	4	4.5	15.5		8.82	
20	1.301	20	6	7	6.5	13.5		20.59	
40	1.602	20	10	11	10.5	9.5		44.12	
80	1.903	20	14	15	14.5	5.5		67.65	
160	2.204	20	17	18	17.5	2.5		85.29	
320	2.505	20	20	19	19.5	0.5		97.06	**39.273**

4.6 Result
The brine shrimp lethality bioassay demonstrated that the ethanolic extract of Eclipta prostrata leaves exhibits significant cytotoxic potential with an LC_{50} value of 39.273 µg/ml. This value indicates strong biological activity, particularly when compared to the standard drug vincristine sulfate, which exhibited an LC_{50} of 0.738 µg/ml [21, 22].

4.7 Discussion
The cytotoxic potential observed in the *Eclipta prostrata* leaf extract confirms the presence of bioactive constituents capable of inducing lethality in Artemia salina nauplii. The assay showed a clear dose-dependent increase in mortality, indicating a probable pharmacological mechanism. The LC_{50} value of 39.273 µg/ml suggests considerable cytotoxic potential, aligning with previous findings on the biological activity of this plant. These results support the traditional uses of Eclipta prostrata and provide a basis for further isolation and characterization of its bioactive compounds.

5. Evaluation of *in-vitro* Anticancer Activity
Ex-vivo culture of established cell lines contributes largely in the mechanistic studies of human cancer. The overwhelming majority of the studies are performed using immortalized cell lines cultured on two-dimensional plastic substrate. The first human cancer cell line was established in 1951 and was derived from a cervical tumor. These HeLa cells were the first cell line capable of indefinitely propagating in an artificial environment. The first pancreatic cancer–derived cell line was reported in 1963 and named the CaPa strain. Cell cultures have been used to study pathophysiological and differentiation processes in cells for decades and hold some great advantages. They are easy to culture, grow indefinitely in cell growth medium, can be cryopreserved, bypass ethical issues associated with animal and human experiments, and can be genetically and chemically altered in a controlled environment. In addition, large drug screens can be performed using cell lines allowing for more insights into the effects and working mechanisms of drugs. Since the mid-1980s, panels of human cancer cell lines grown in-vitro have represented the workhorse of cancer drug screening. This is largely accounted for by the empirical-based approach adopted at this time by the NCI. The NCI abandoned its use of screens involving rapidly growing mouse leukemia lines *in vivo* for a panel of 60 human tumor cell lines derived from 9 tumor types [23].

Cervical cancer was the second highest diagnosed and the third leading cause of cancer death in females in ASEAN countries [24]. It accounted for 11% of total new cancer cases and 9% the total cancer deaths among female in 2008 respectively. More than 90% of cervical cancer cases arise as a consequence of a human papilloma virus (HPV) infection [25]. Currently, 210 different HPV types have been officially recognized. Some cervical cancer cell lines that are used are the following: HCK1T (Human Cervical Keratinocytes), a normal cervical epithelium cell line; HeLa, a cervical cancer cell line positive for HPV18; SiHa, a cervical cancer cell line positive for HPV16; and C-33A, a cervical cancer cell line negative for HPV. A HeLa cell is an immortal cell line in fact the first human cancer cell line used in medical research. This cell line was derived from cervical cancer.

5.1 Materials and Methods
5.1.1 Reagents
- Adherent cell line of interest (HeLa)
- Appropriate culture medium- DMEM (Dulbecco's Modified Eagles' medium)
- NaHCO3 (Sigma, cat. no. S5761)
- 10 mM minimal essential medium (MEM) non-essential amino-acid solution
- MEM (Eagle) supplemented with 2 mM L-glutamine and Earle's balanced salt solution (EBSS)
- 100 mM sodium pyruvate
- Fetal bovine serum (FBS)
- 10 mg ml–1 bovine insulin in 25 mM HEPES, pH 8.2
- 2.5% (wt./vol.) trypsin solution
- 0.5% (wt./vol.) phenol red solution
- 0.48 mM versene-EDTA
- 0.4% (wt./vol.) trypan blue in 0.81% (wt./vol.) NaCl and 0.61% (wt./vol.)
- KH2PO4
- Dimethyl sulfoxide (DMSO)
- Positive control: Doxorubicin
- 10% (wt./vol.) TCA
- 1% (vol./vol.) acetic acid
- 10 mM unbuffered Tris base solution

- Antibiotics (penicillin+streptomycin)

5.1.2 Equipments

- 96-well clear flat-bottom polystyrene tissue-culture plates
- 75-ml tissue-culture flasks or 100 mm tissue-culture plates
- Biological Bio Safety Cabinet (Model: NU-400E, Nuaire, USA)
- CO2 incubator (Nuaire, USA)
- Inverted microscope (TMS, Nikon)
- Multi-well microplate reader (Wallac Victor V, PerkinElmer)
- Multi-channel pipette (Gilson)
- Gyratory plate shaker (Model 4625, Lab-Line)
- Hemocytometer

5.2 Procedure

5.2.1 Compound preparation

- Dissolving dried sample (*Eclipta prostrata* leaf extract) with 5% (vol./vol.) DMSO to 10 mg ml–1.
- For primary screening, dilute the dissolved compound with sterile 5% DMSO. For IC50 determination, make a twofold serial dilution from 1 mg ml^{-1} to 16 µg ml^{-1} in 5% (vol./vol.) DMSO. Mix compound solution by pipetting thoroughly several times after each transfer. (**Critical step:** To prevent attachment of the compound to the plastic ware, we used polypropylene micro-centrifuge tubes or 96-well plates for compound dilution. Pipette tips should be changed after each transfer).

Cytotoxic effect was examined at **Centre for Advanced Research in Sciences, Dhaka University** using their commercial services. In brief-

- HeLa, a human cervical carcinoma cell line was maintained in DMEM (Dulbecco's Modified Eagles' medium) containing 1% penicillin- streptomycin (1:1) and 0.2 % gentamycin and 10% fetal bovine serum (FBS).
- HeLa cells (2×10^4/100 µL) were seeded onto 96-well plate and incubated at 37° in 5% CO2 incubator.
- Next day 25 µL of sample (filtered) was added to each well (1024 µg/ml, 512 µg/ml, 256 µg/ml, 128 µg/ml and 64 µg/ml).

Cell viability was examined after 48h of incubation using a colorimetric cell proliferation assay kit (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega, USA) following the manufacture's protocol. Duplicate wells were used for each sample (**Insert table 5.1 here**).

5.3 Result

Table 5.1: Effect of ethanolic extract of *Eclipta prostrata* leaf on human HeLa cervical cancer cells

Test extract Conc. (µg/ml)	Cell Viability (% of control) in HeLa cell line	LD50
64	84.11	1505 mg/ml
128	78.85	
256	72.12	
512	67.52	
1024	62.09	

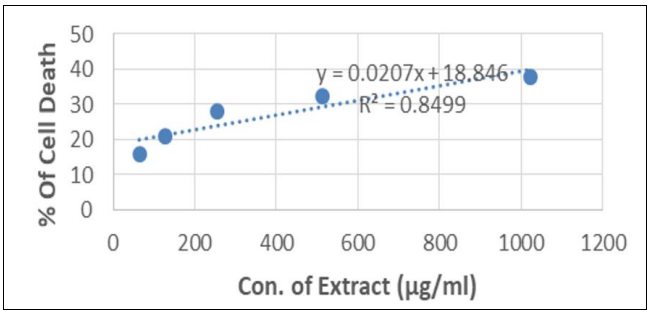

Fig 5.1: Effect of ethanolic extract of *Eclipta prostrata* leaf on human HeLa cervical cancer cells

5.4 Discussion

The test extract dose dependently reduced cell survival in the cells (**Table 5.1** and **Figure 5.1**). The IC50 value for the test extract is >1024 µg/ml. [26] The obtained IC50 value for the ethanolic extract of *Eclipta prostrata* leaf suggests its moderate cytotoxicity against HeLa cancer cell lines that prompted its further assessment as a chemopreventive agent in an *in-vivo* animal model.

6. Conclusion

This study systematically investigated the toxicological and cytotoxic profiles of ethanolic extract of Eclipta prostrata leaves. Following plant identification and extraction, acute and chronic toxicity studies in mice confirmed the extract's safety, with no mortality or toxic symptoms observed even at high doses. Biochemical and hematological analyses remained within normal ranges throughout the 150-day chronic exposure. The brine shrimp lethality bioassay demonstrated significant dose-dependent lethality, reflecting strong cytotoxic potential. In-vitro assays against HeLa cervical cancer cells revealed a moderate growth inhibitory effect, supporting the traditional use of E. prostrata in cancer-related therapies. The results suggest that E. prostrata leaf extract is safe for medicinal use and a potential source of anticancer agents.

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