Evaluation of Anticancer, Anthelminthic, Anti-nociceptive, Antidiabetic and Toxicological Investigation of *Ludwigia adscendens*

ABSTRACT

The present research investigated *Ludwigia adscendens* crude methanol extract invitro anticancer anthelminthic, and invivo anti-nociceptive, antidiabetic and toxicological properties. The coarsely dried plant powder was extracted using methanol. The methanolic extract (MELA) was further tested for anticancer, anthelminthic, anti-nociceptive, antidiabetic and toxicological activities. Cell Viability Assay was used for anticancer testing, and the earthworm assay was used for anthelminthic testing using different concentrations. Antinociceptive tests were done on Swiss albino mice at 200 and 400 mg/kg utilizing a hot plate, acetic acid induced writhing & formalin-induced paw licking tests. Antidiabetic test was done using Blood Glucose Determination test using the dose of 150 mg/kg and 300 mg/kg. Acute toxicity was tested utilizing cinnamon oil-induced toxicological tests at 3000, 5000 and 7000 mg/kg. The MELA demonstrated 39.16% inhibition at 1000µg/mL in the Cell Viability Assay. The earthworm died after 6 minutes and 4 seconds in the 100 mg/mL anthelminthic test, whereas Albendazole killed it in 4 minutes and 20 seconds. Hot plate test results were substantial. The formalin-induced nociception test demonstrated strong inhibition rates of 79.54% in the early phase and 74.54% in the late phase at 400 mg/kg, compared to 62.99% and 68.18% for diclofenac sodium. Acetic acid-induced writhing test showed 77.66% of pain inhibition where's Diclofenac sodium showed 79.61%. MELA inhibited blood glucose level very significantly compared to the standard Glibenclamide. In toxicological testing, 7000 mg/kg killed mice 2/5, whereas cinnamon oil killed 5/5 within 24 hours. The study shows that MELA has moderate anticancer, significant anthelmintic, anti-nociceptive, antidiabetic and mild toxicological properties. They may support the plant's use in conventional medicine to relieve pain, minimize drug intoxication, and prevent cancer, control diabetes and parasitic disorders.

Keywords: *Ludwigia adscendens*, anticancer, antidiabetic, anthelmintic, HeLa cell.

1. INTRODUCTION

The influence of traditional medicine on modern medical practices has been substantial. Many modern medications, including aspirin and quinine, are derived from plants that were utilized in ancient medicine [1]. Furthermore, it is worth noting that traditional medicine serves as a significant asset in the exploration of innovative pharmaceutical compounds [2]. In addition, traditional medicine plays a significant role in modern healthcare by providing cost-effective and easily available alternatives to pharmaceutical medications. The use of medicinal plants has had a substantial impact on the advancement of therapeutic interventions for many health disorders. Phytochemicals produced from medicinal plants have shown promise in the field of cancer treatment, exhibiting potential in both cancer prevention and treatment. An

example of an anticancer drug is paclitaxel, which is obtained from the bark of the Pacific yew tree, *Taxus brevifolia*. This medicine has shown efficacy in the treatment of cancer [3]. Medicinal herbs have been essential in the treatment of parasitic worm illnesses via anthelmintic therapy. Numerous conventionally used medicinal herbs, such as *Carica papaya* and *Azadirachta indica* (neem), have shown encouraging anthelmintic properties [4]. Medicinal herbs have made a substantial contribution to the creation of anti-nociceptive medications, which are used to treat pain. One such is the medication morphine, which has long been a mainstay in pain treatment and is produced from the opium poppy plant, *Papaver somniferum*[5]. Lastly, the treatment of diabetes, a disease marked by increased blood glucose levels, has also benefited greatly from using medicinal herbs. For instance, a traditional medicinal herb called *Gymnema sylvestre* has been shown to have anti-diabetic effects by boosting insulin production, which helps to regulate blood sugar levels [6].

Ludwigia adscendens, also known as floating primrose-willow, is a species of flowering plant in the family Onagraceae. It's native to the Americas but has spread to other continents including Africa and Asia, where it is often considered an invasive species [7].The plant is notable for its medicinal properties. In traditional medicine, *Ludwigia adscendens* has been used to treat various ailments, including gastrointestinal disorders, respiratory illnesses, and skin disease[8]. Recent scientific studies have validated some of these uses. For instance, a study found that extracts of *Ludwigia adscendens* demonstrated antimicrobial activity, lending scientific support to its traditional use in treating skin infections [9]. *Ludwigia adscendens* is also used in environmental management. Due to its rapid growth and floating nature, it is used in constructed wetlands for wastewater treatment, where it helps to remove pollutants such as heavy metals from contaminated water [10].The purpose of this study is to screen for phytochemicals and ascertain the anticancer, anthelminthic, anti-nociceptive, antidiabetic, and toxicological activities of *Ludwigia adscendens* using its methanolic leaf extract.

2. MATERIAL and METHOD

2.1 Plant Material

In October 2022, a sample of *Ludwigia adscendens* was collected from West-Delpara, Kutubpur, Narayanganj, Dhaka. The plant was successfully identified by the specialists at the Bangladesh National Herbarium in Mirpur, which is situated in Dhaka (Accession number: DACB 87895). After plant accession, the whole plant was crushed into a fine powder and dried for 11days in the shade in preparation for conducting pharmacological tests.

2.2 Preparation of the Methanolic Plant Extract

After the dirt was removed, the whole fresh *Ludwigia adscendens* plant was retrieved. Then, to get rid of all the dust, the whole plant was cleaned in room temperature water. Washing was followed by a 12- to 15-day air drying period in the shade for *Ludwigia*

adscendens. The plant was broken up into small pieces and mixed by a grinder machine after it had thoroughly dried. For three days, 66g of powdered *Ludwigia adscendens* were steeped in as much as three fingers of methanol. Stirring was done occasionally to aid in the maceration process. The filter paper was used to filter the extract after three days. After the solvent was allowed to air dry, 6.79g of extract was produced. The raw extract was stored in a beaker, kept cold, and kept out of direct sunlight.The whole protocol was followed from previous research [10].

2.3 Phytochemical Screening Test

The medicinal properties of plants are derived from their chemical components. The freshly generated MELA was qualitatively evaluated using different reagents for the presence of phytochemicals such as alkaloids, carbohydrates, saponins, glycosides, reducing sugar, flavonoids, tannins, and steroids[11]

2.4 In-vitro Analysis

2.4.1 Anticancer test

- **Cell viability assay**

The potential anticancer effect was investigated using HeLa cells. A human cervical carcinoma cell (HCT-116) was cultured in DMEM (Dulbecco's Modified Eagles' medium) supplemented with 1% penicillin-streptomycin (1:1), 0.2% gentamycin, and 10% fetal bovine serum (FBS). HeLa cells were seeded onto a 96-well plate and incubated at 37°C with 5% CO2, just like a pharmacologist would do. The following day, a 25 µL filtrated sample (MELA) was added carefully to each. The cell viability was assessed after 48hours of incubation using the CellTiter 96 Non-Radioactive Cell Proliferation Assay kit from Promega, USA. Each sample was tested using duplicate wells[12].

- **Morphology study**

Cells were plated in 24-well plates and subjected to treatment with either DMSO or extract at the IC_{50} concentration for 24 hours. Following the treatment, the image was captured using phase contrast microscopy [12].

2.4.2 Anthelminthic test

The worm motility inhibition test was used to assess MELA's anthelmintic activity in vitro against adult *H. contortus*. Before being delivered to the lab, the worms were washed and then put in phosphate-buffered saline (PBS). MELA was assessed at a 25 mg/ml concentration after being dissolved in 0.5% dimethyl sulphoxide (DMSO). As the positive control, 0.55 mg/ml of the well-known anthelmintic albendazole was dissolved in DMSO (0.5%). DMSO at a 0.5% concentration served as the negative control. Each treatment was applied to twenty worms at a controlled temperature of 35±1°C. There were three copies of every therapy. The anthelmintic therapy was beneficial because it decreased worm movement. There were intervals of 0, 1, 2, and 4 hours in which the times for paralysis, complete inactivity, and death were recorded. To evaluate the

recovery of worm motility, the extracts and albendazole were withdrawn after 4 hours, and the parasites were resuspended in lukewarm PBS for 30 minutes[13].

2.5 In-vivo Experiments

2.5.1 Experimental animals

For this whole research, young, healthy *Swiss albino* mice weighing between 22 and 25g were used. These mice were collected from Jahangirnagar University in Dhaka, Bangladesh, at their Saver facility. A temperature of 77°F, a relative humidity of 55 to 65%, and a 24-hour cycle of light and dark are examples of typical atmospheric fluctuations. After collection, circumstances remain unchanged for eight days. To help mice recover from the water and food shortage they encountered during transit and adjust to the lab setting, a diet consisting of sufficient food and hygienic water was provided, following the guidelines provided by Jahangirnagar University. The mice recovered for ten days before the experiment was conducted.

2.5.2 Antinociceptive test

- **Hot plate test**

The hot-plate test (Eddy's hot plate) was used to assess the analgesic activity, as stated by previous research [14], [15]. The thermostat was programmed to maintain a temperature of 51°± 1°C. Male and female mice were divided into four groups, each containing five mice. To evaluate each group's mice's response to an electrical heat-induced pain stimulus, they were placed in a beaker on a hot plate. Licking of the paws was noted as one of the animal's reactions to the excruciating heat. By timing how long it took each mouse to lick its paws or climb out of the beaker, their reaction times (in seconds) were calculated. Before any kind of treatment was administered, the response time was assessed once. The mean of this decision was used to determine each mouse group's initial reaction time before treatment. After that, oral doses of distilled water (DW), Diclofenac sodium (10mg/kg BW), and MELA (250 and 500 mg/kg BW, respectively) were given to each test mouse. In each mouse group, reaction times were tested five times at one-hour intervals starting thirty minutes after treatment was administered. The formula for the analgesic effectiveness of treatment was as follows:

Percent Analgesic Score = $\frac{Ta-Tb}{Ta}$ X100.

Time (in seconds) to react (before medication administration): Tb; Time (in seconds) to react (after drug administration): Ta.

- **Formalin Induced paw licking test**

The formalin test produces two distinct stages of increased licking activity that are attributed to distinct nociceptive pathways, making it a valid and trust worthy model of nociception. After the formalin injection, there are two phases of licking: the early phase

lasts for the first five minutes, and the late phase occurs 15 to 45 minutes later. As mentioned before, the right hind paw's dorsal surface received a subcutaneous injection of formalin (20µL of a 2.5%solution). After that, the animals were placed on a glass surface under a glass funnel, and a 45-degree-angled mirror was used [16]. The pain response time (licking time) was measured in two phases: the first phase, which lasted 0 to 5 minutes and was brought on by the nociceptors directly, and the second phase, which lasted 15 to 45 minutes and was triggered by the release of inflammatory mediators and resulted in inflammatory pain [21]. Five groups (n = 5) of animals were randomly assigned to them. The negative control group's animals were given 0.5 mL of regular saline. Animals in the positive control group were treated with morphine (10 mg/kg, Temad Co., Iran). MELA was administered at varying levels (250 and 500 mg/kg) to the other groups. All injections were administered intraperitoneally 30 minutes before the test [17].

- **Acetic acid induced writhing test**

With minor adjustments, the acetic acid-induced writhing methodology in mice was used to test the analgesic activity of the sample. This method is comparable to that used by previous research work [18]. The experiment's animals are given an intraperitoneal injection of acetic acid, which causes them to suffer. To investigate the animals, four groups of five mice each were used. After an overnight fast, animals in Group I received distilled water, those in Group II received 10 mg/kg of Diclofenac sodium, and those in Groups III and IV received 250 and 500 mg/kg of MELA, respectively. The test samples and vehicle were administered orally thirty minutes before the intraperitoneal injection of a 0.7% v/v acetic acid solution. To enable researchers to examine the animals, each was kept in a separate transparent glass container. The acetic acid solution was given intraperitoneally to the mice five minutes later. For the following ten minutes, we counted the number of times each mouse in each group writhed in its cage. Not every animal writhed in its entirety; others would just begin. It was found that this kind of writing was only partially finished. Thus, one entire writhing was counted as two half-ones. The number of writhing in each group was compared to a positive control group that was given a placebo consisting of Diclofenac sodium. The proportion of writhing restraint was determined by using the following formula:

$$\% \text{ Of writhing} = \frac{VC - VT}{VC} X\ 100$$

VT = number of writhing motions in extract-treated mice. VC = number of writhing motions in the control group of mice.

2.5.3 Antidiabetic Test

- **Induction of hyperglycemia**

186.9 mg/kg of a 10% alloxan monohydrate solution was administered intraperitoneally to the subjects. The alloxan monohydrate was procured from Sigma in Switzerland.

After 48 hours of alloxan administration, blood glucose levels were assessed using a glucometer. Mice with blood glucose levels over 200mg/mL were classified as diabetic and included in the research. Before starting the experiment, the animals were fasted for 8-12 hours but were permitted to drink water during the trial [19], [20].

- **Experimental design**

Eight groups were created, with five mice in each group, to administer medication intraperitoneally or orally. Group I consisted of regular mice administered 0.1 ml of physiological saline either through intraperitoneal injection or oral ingestion. Group II consisted of alloxan-induced diabetic mice that were treated like the first group, using 0.1 ml of physiological saline. Group III consisted of alloxan-induced diabetic mice who received 0.025 insulin units (1 IU/kg body weight) intraperitoneally in 0.1 ml physiological saline. Group IV received an oral administration of 0.075 mg glibenclamide (3mg/kg body weight) in 0.1 ml physiological saline to alloxan-induced diabetic mice. Groups V, VI, and VII included alloxan-induced diabetic mice that received different doses based on their body weight. The doses were administered either intraperitoneally or orally in 0.1 ml physiological saline. Group VIII consisted of alloxan-induced diabetic mice who were given different treatments: insulin, glibenclamide, or a plant extract solution. The dosage was 350 mg/kg body weight in 1 ml of physiological saline, administered either intraperitoneally or orally.

- **Blood glucose determination**

The blood sample included sterilizing the tail with 10% alcohol and then pricking it at the beginning of the experiment, and repeating this process after 1, 2, 3, 4, 6, and 24 hours. Enhanced bleeding by slowly expressing blood from the tail towards the tip. Following the surgery, the tail tips were sterilized by swabbing them with 70% ethanol. The blood glucose levels were measured using a glucose analyzer model (Hypoguard, Wood bridge, England).

2.5.6 Acute toxicological test

Each group had five mice that received oral dosages of MELA and cinnamon oil at 1000 mg/kg, 2000 mg/kg, or 3000 mg/kg; water was used as the control. After a 24-hour observation period, the death rates for both groups were noted[21].

3. STASTICAL ANALYSIS

The experimental data was replicated three times, and the mean and standard deviation were utilized to represent the results. Excel is also used for statistical studies.

4. RESULTS

4.1 Phytochemical Identification

MELA was subjected to thorough phytochemical analysis, revealing a diverse range of phytochemicals including tannin, flavonoids, saponin, reducing sugars, alkaloids, gums, glycosides, steroids, and phenolics, among others. However, Table 1 did not contain any carbohydrates.

Table 1. Results of phytochemical screening test of MELA

Phytoconstituents	MELA
Steroid	+
Alkaloid	+
Saponin	+
Phenolics	+
Carbohydrates	–
Tannin	+
Glycosides	+
Gum	+
Reducing sugar	+
Flavonoid	+

4.2 Anticancer activity

The standardization of plant materials alcoholic extract (MELA) was done in accordance with accepted practices, and the extract's anticancer potential was tested on HeLa cell lines. The methanolic extract from the *Ludwigia adscendens* plant demonstrated this potential (Table 2).

Table 2. Anticancer Activity of MELA.

Concentration (µm/mL)	Survival of the cell (%)	% of Inhibition
125	85.69	14.31
250	76.94	23.06
500	69.63	30.37
1000	60.84	39.16

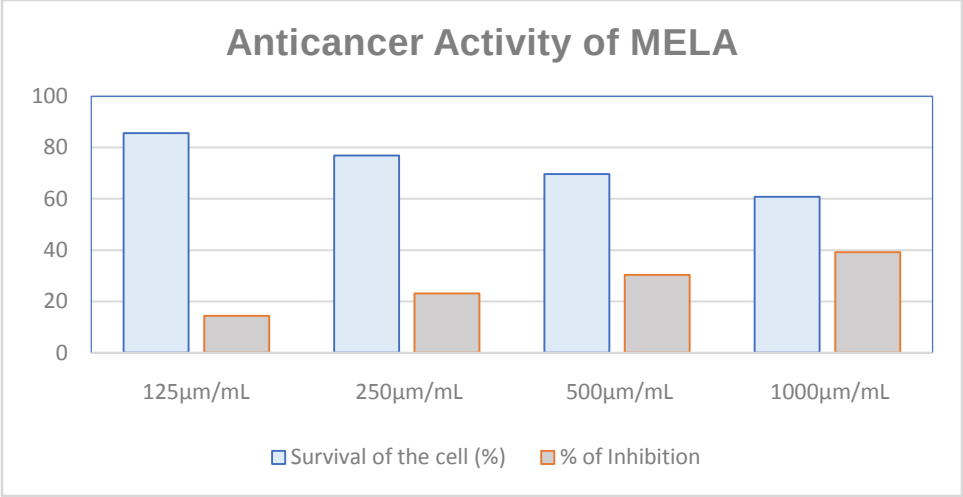

Figure 1. Graph of anticancer activity of MELA.

4.3 Anthelmintic activity

In order to evaluate the anthelmintic effectiveness of fresh leaf juice, earthworms were subjected to several amounts of the liquid (5mg/mL, 10mg/mL, 20mg/mL, 50mg/mL, and 100mg/mL). Comparable to the standard medication albendazole, the leaf extract exhibited strong anthelmintic activity (Table 3).

Table 3. Anthelmintic Activity of MELA

Test samples	Conc. (mg/mL)	Time taken for paralysis	Time taken for death
MELA	5	64 min 52 sec	70 min 09 sec
	10	57 min 20 sec	62 min 53 sec
	20	54 min 28 sec	58 min 33 sec
	50	34 min 51 sec	37 min 47 sec
	100	25 min 11 sec	29 min 54 sec
Albendazole	5	76 min 09 sec	82 min 19 sec
	10	62 min 47 sec	72 min 32 sec
	20	57 min 52 sec	69 min 37 sec
	50	41 min 03 sec	47 min 12 sec
	100	30 min 43 sec	38 min 18 sec

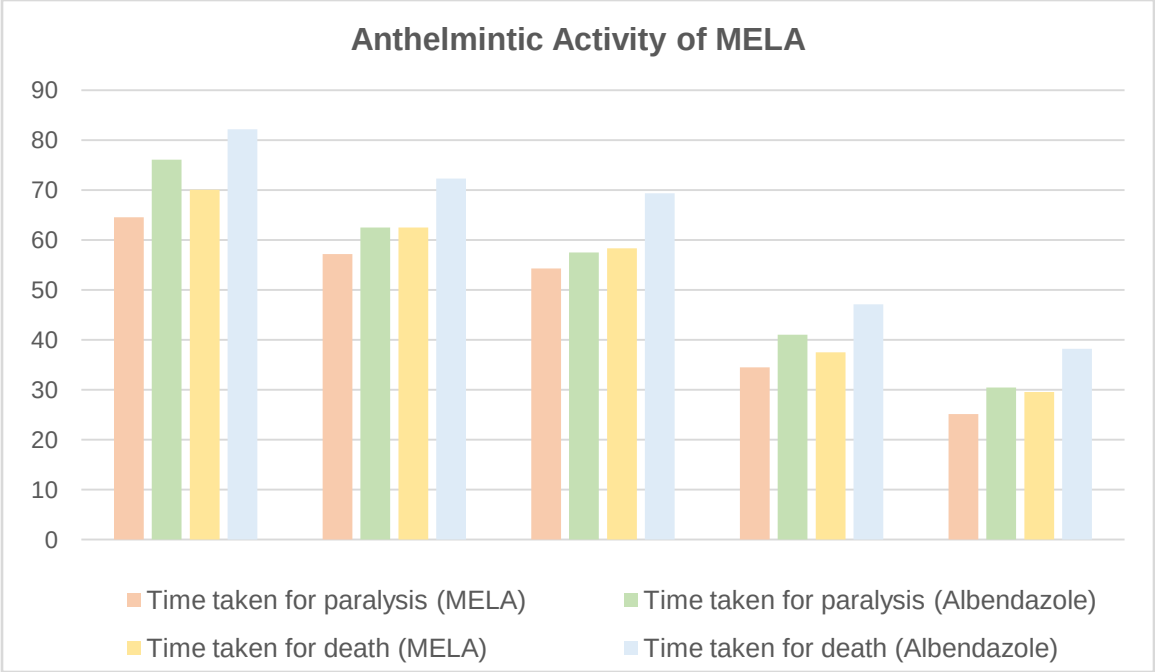

Figure 2. Graph of anthelmintic activity of MELA

4.4 Antinociceptive activity

4.4.1 Hot plate experimental activity

The results of the methanol leaf extract of *Ludwigia adscendens on* mean reaction time in the hot plate test can be found in Table 4.The extract at the doses tested; significantly increased the latency to response in a dose-dependent manner.

Table 4Antinociceptive effect of leaf extract of *Ludwigia adscendens* on hot plate test

	Reaction time at different time intervals (in sec)			
Group	30 min	60 min	90 min	120 min
Control	6.4	7.6	6.0	5.4
Morphine (5mg/kg)	8.6	9.4	11.0	16.6
MELA (250mg/kg)	5.8	8.2	11.5	0
MELA (500mg/kg)	7.7	9.5	17.6	0

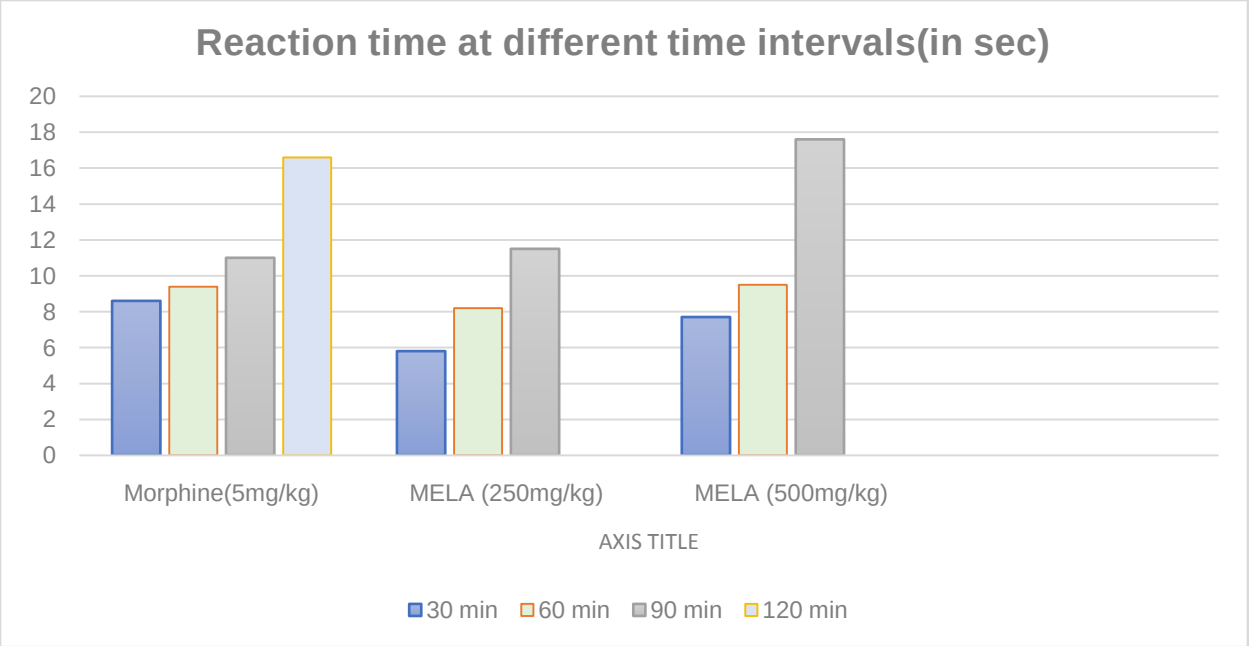

Figure 3. Graph of antinociceptive effect of MELA using hot plate method.

4.4.3 Formalin Induced nociceptive activity

Based on the results presented in Table 5, it is evident that during the nociceptive phase (early phase), the administration of MELA (250 mg/kg) resulted in a significant increase in nociceptive response compared to the control group .In the late phase (phaseII), MELA at 250 and 500 mg/kg demonstrated a highly notable antinociceptive effect.

Table 5. Antinociceptive effects of MELA in formalin-induced nociception

Treatment	Dose (mg/kg)	Licking of the hind paw			
		Early phase	% of inhibition early Phase	Late phase	% of inhibition late phase
Control	0.1mL/mice	25.40	0	13.20	0
Diclofenac sodium	10	9.40	62.992	4.20	74.182
MELA	250	20.60	18.898	9.40	68.88
MELA	500	14.00	44.882	6.80	78.485

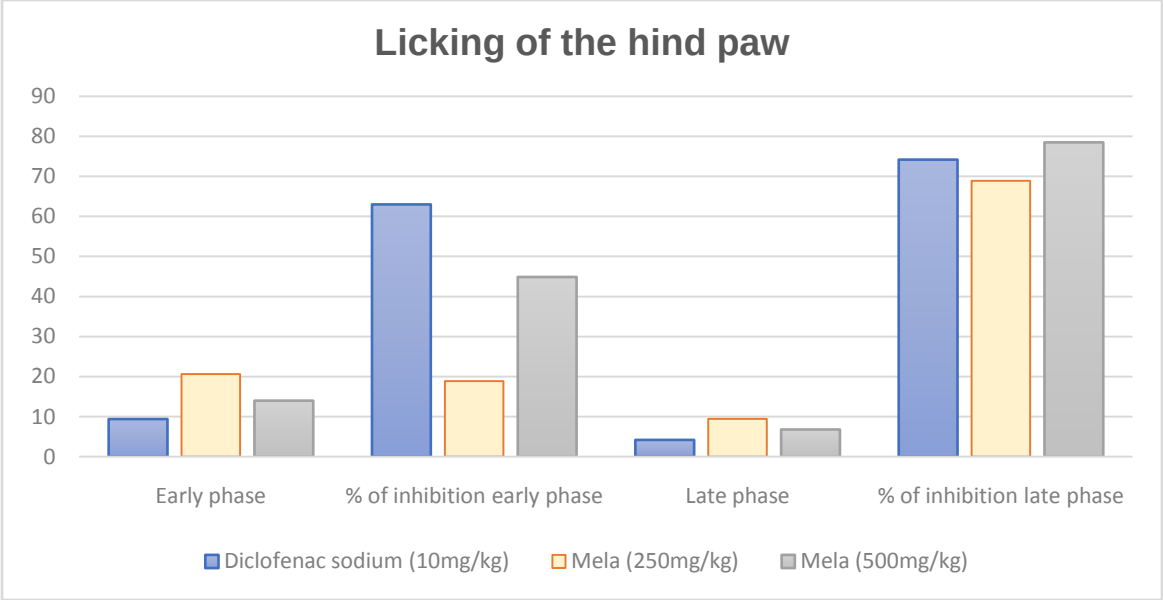

Figure 4. Graph of antinociceptive effect of MELA using formalin Induced nociception method.

4.4.4 Acetic acid induced nociceptive

The results from the research on the impact of MELA on the acetic acid-induced writhing reflex in mice are displayed in Table 6. The extract demonstrated a noteworthy dose-dependent decrease in the number of writhing reflexes in the treated mice, in comparison to the negative control group. Using different substances, pain inhibition of 79.61%, 70.52%, and 77.66% was observed in experimental mice.

Table 6: Effect of MELA on acetic acid-induced writhing reflex.

Administered Substance	Dose	% Writhing	% Of Inhibition
Control	10mL/kg	100	0.00
Diclofenac sodium	10mg/kg	20.40	79.61
MELA	200mg/kg	29.48	70.52
MELA	400mg/kg	22.34	77.66

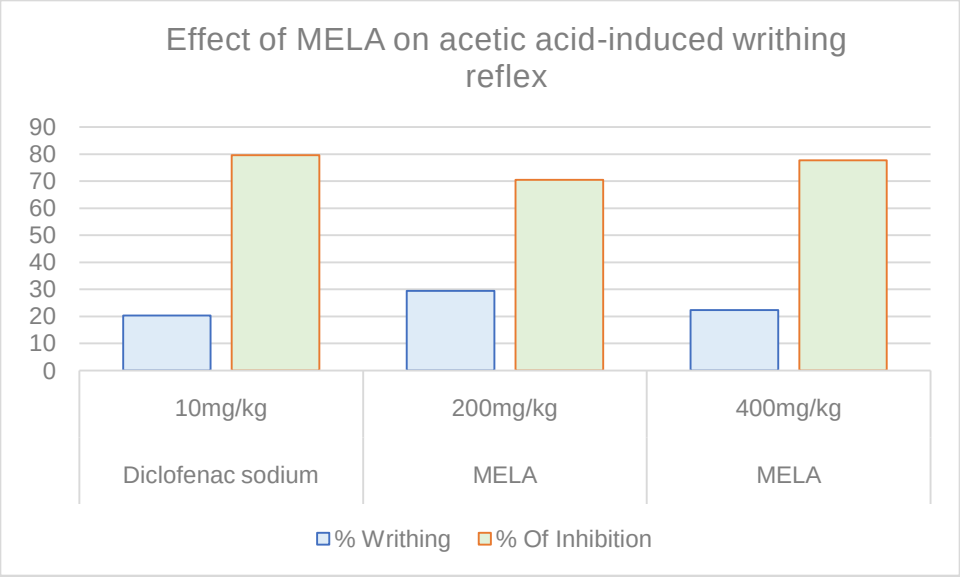

Figure 5. Graph of antinociceptive effect of MELA using acetic acid Induced writhing test.

4.5 Antidiabetic activities

The research convincingly shows that MELA, specifically MEPH at doses of 150 mg/kg and 300 mg/kg, has a notable impact on reducing blood glucose levels in diabetic mice. According to Table 7, there is promising evidence that MEPH could be used as a treatment for managing diabetes. It appears that a higher dosage of MEPH has a stronger impact on the condition.

Table 7. Effects of intraperitoneally administered MELA on blood glucose levels in alloxan induced diabetic mice

Test Samples	Blood Glucose Levels at Varying Times (mmoles/L)			
	½ hour	1 hour	2 hours	3 hours
Control	5.21±0.05	5.31±0.11	5.33±0.03	5.28±0.07
Diabetic Saline	14.53±0.11	16.02±0.15	17.51±0.06	18.52±0.03
Standard Glibenclamide	17.43±1.30	8.51±0.07	6.21±0.42	4.96±0.11
MELA (150 mg/kg)	11.57±0.21	9.31±0.02	6.56±0.33	5.03±1.36
MELA (300/mg/kg)	12.26±0.31	7.56±0.15	5.48±1.05	4.03±0.26

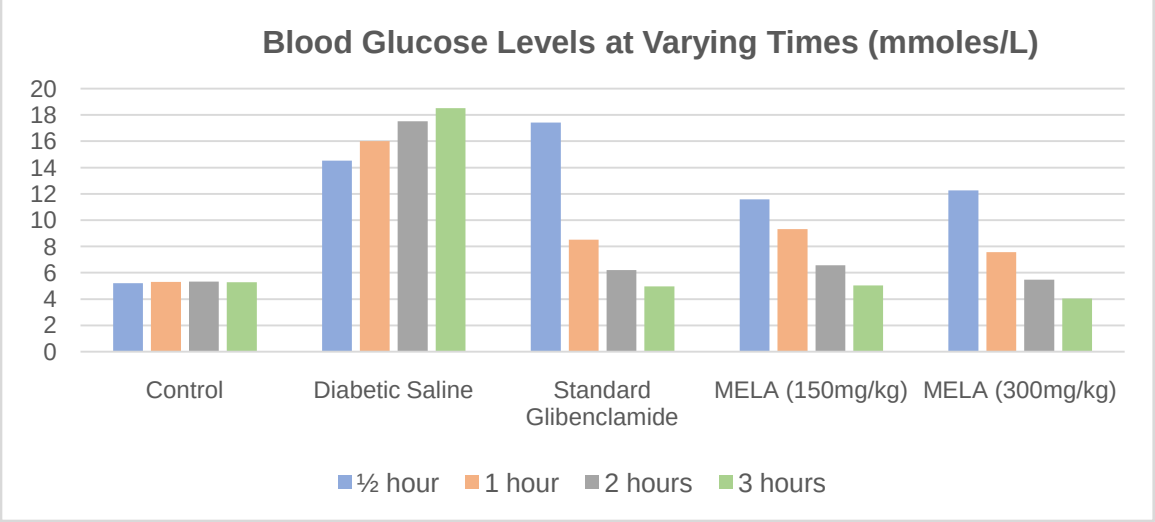

Figure 6. Graph of antidiabetic effect of MELA.

4.6 Toxicological activities

Throughout the observation period, oral administration of MELA at 1000 and 3000 mg/kg doses did not cause any deaths, allergic reactions, excessive drooling, seizures, tremors, diarrhea, or abnormal behavior. In addition, there were no statistically significant macroscopic alterations or abnormalities observed in the important organs of the mice between the control and experimental groups. However, when administering a dose of 5000 mg/kg of MELA, the experimental mice experienced mortality.

Table 8. Results of Acute toxicological activities of MELA

Sample	Onset time of seizure (s)	Mortality protection after 30min	Mortality protection after 24h
Control (Normal saline)	27±2.91	0/5	0/5
Cinnamon oil (20mg/kg)	342±3.72	5/5	5/5
MELA (1000mg/kg)	18±1.29	0/5	0/5
MELA (3000mg/kg)	73±1.04	0/5	0/5
MELA (5000mg/kg)	114±1.82	1/5	2/5

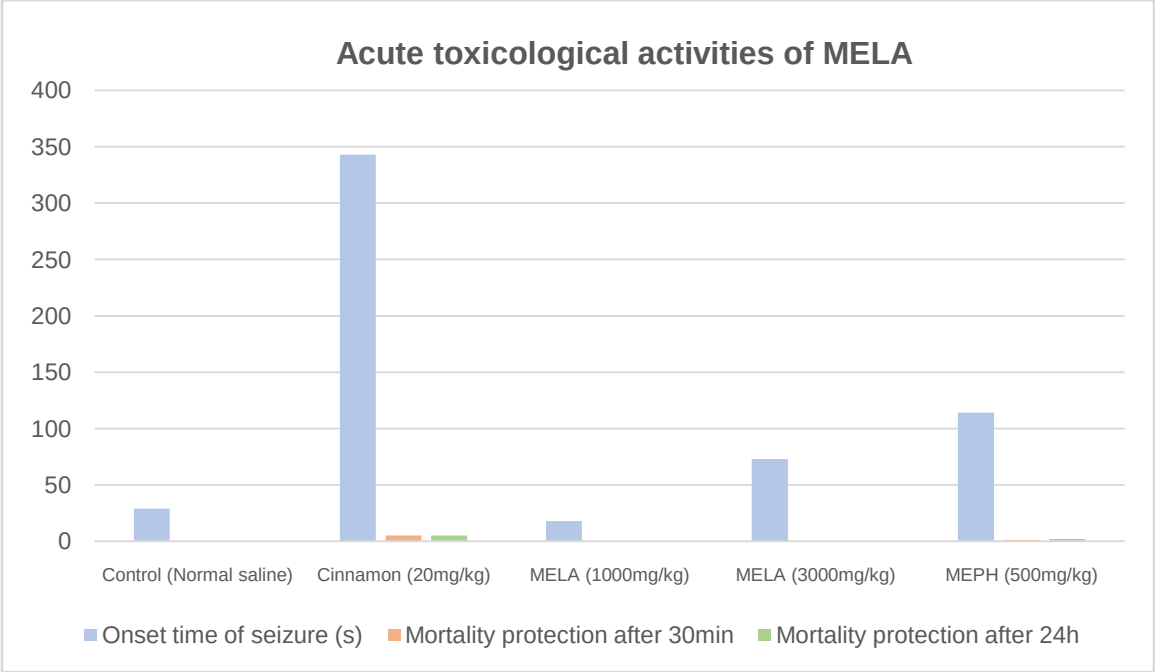

Figure 7. Graph of acute toxicological activities of MELA

5. DISCUSSIONS

The current research is aimed to assess the effects of MELA on various pharmacological tests, such as anticancer, anthelminthic, anti-nociceptive, antidiabetic, and toxicological activities. In addition, the chemical composition of MELA was analyzed through phytochemical screening. The study's findings, as shown in Table 1, reveal the presence of various compounds in MELA, such as tannin, flavonoids, saponin, reducing sugars, alkaloids, gums, glycosides, steroids, and phenolics. Interestingly, MELA did not contain any carbohydrates. However, these present phytochemicals have a wide range of pharmacological properties, including analgesic, anti-cancer, antioxidant, antimicrobial, anticonvulsant, anthelmintic, anti-inflammatory, and cytotoxic effects[22].

The occurrence of colon cancer has been steadily rising due to recent lifestyle changes, such as a diet low in vegetables and fruits, lack of physical activity, excessive alcohol consumption, and exposure to harmful chemicals [23]. Despite the advancements in routine check-ups and early detection, colon cancer continues to claim a significant number of lives worldwide each year. There is a pressing need to discover new therapeutics or drug candidates that can selectively target cancer cells while leaving normal cells unaffected [24].

Here, this study investigated the potential anticancer effects of the methanol extract MELA derived from *Ludwigia adscendens* on human cancer cells. Cancer cells have a higher basal level of reactive oxygen species (ROS) due to their increased metabolic rate and other unique functions, which sets them apart from normal cells. An increased level of reactive oxygen species (ROS) is crucial for the growth, proliferation, and metastasis of cancer cells. Simultaneously, an excessive amount of ROS beyond what is necessary can induce oxidative stress in cancer cells, potentially resulting in their demise. In contrast to normal cells, cancer cells have a limited antioxidant mechanism to scavenge excess ROS and prevent cellular damage [25]. It is conceivable that the extract's anticancer activity observed in this study is a result of compounds that have modified the redox balance, which is crucial for the survival of HCT-116 cells. This activity may either increase or decrease the level of reactive oxygen species (ROS) in HCT-116 cells [26]. The minimal toxicity of MELA towards normal cells is likely attributed to the robust antioxidant and anti-inflammatory mechanisms found in these cells [25].The findings presented in this study offer reliable evidence that the methanolic extract of *Ludwigia adscendens* contains potential anti-colon cancer compounds. These compounds show promise for further investigation to understand better and explore their potential for therapeutic development.

The discovery of resistant strains, the detection of drug residues in animal products, and the potential toxicity of synthetic drugs have sparked renewed interest in the utilization of natural products [27]. The plant materials tested for their in vitro anthelmintic activity in this study were identified by local livestock raisers. In the present in vitro study, a concentration of 10 mg/mL of methanol extract from *Ludwigia adscendens* demonstrated a statistically significant anthelmintic activity, similar to that of the

conventional anthelmintic agent, albendazole. In addition, the genus contains a wide variety of sesquiterpene lactones and flavonoids that could potentially exhibit anthelmintic properties while posing minimal risk of toxicity to mammals [28]. The observed anthelmintic effect of MELA could potentially be ascribed to the presence of secondary metabolites. Prior research [29] has indicated that tannins might exhibit anthelmintic activity by inhibiting hatching, impeding development into the infective larval stage, and reducing adult motility. In addition, studies have demonstrated that tannins can disrupt the process of oxidative phosphorylation and inhibit ATP synthesis in *H. contortus*. Another study [30] has further validated the effectiveness of alkaloids derived from plants as anthelmintics. The release of enzymes by larvae is triggered by environmental stimuli, resulting in the degradation of the egg membrane [29]. There could be a connection between the activity of alkaloids in these two plants and the inhibition of these enzymes.

Pain is a physiological response that can be intense and protective. Typically, painful stimuli elicit strong withdrawal and avoidance responses. Given the intricate nature of central nociceptive pathways, which undergo sensitization and rearrangement due to tissue injury and prolonged pain, this experience becomes highly complex [31]. Specialized receptors detect the sensation of pain and are then relayed to the brain via specific neurons and nerves in the spinal cord. The nerves travel through various pathways, connecting the spinal cord to the thalamus in different areas of the brain stem, particularly in the relay nuclei. Third-row neurons transmit sensory pain nerves to various regions of the brain membrane and limbic system. Individuals who have endured chronic pain often face detrimental psychological effects, prompting a relentless pursuit of effective pain management strategies. There have been numerous endeavors to elucidate the mechanisms of pain and strategies for its control. There are two main categories of drugs, synthetic and herbal, that are commonly used to alleviate pain [32]. In this research, it was found that the oral administration of MELA had a significant impact on reducing the threshold for heat and chemically induced pain. Moreover, it exhibited dose-dependent antinociceptive effects in various pain models. Based on our observations in mice, it appears that the doses of MELA used in these experiments did not cause any negative effects such as mortality, allergic responses, salivation, convulsions, tremors, diarrhea, behavioral abnormalities, or physical alterations in important organs. Therefore, it can be concluded that MELA was not found to be hazardous at the levels that were tested.

The nociceptive response to heat stimuli in mice is a well-established model for studying the effectiveness of different types of analgesic medications that target the spinal origin[33].Testing for detection using the hot-plate test using Eddy's hot plate. A pain stimulus is acetic acid. Through the action of certain enzymes, such as phospholipase A_2 and acyl hydrolases, the administration of acetic acid intraperitoneally triggers a specific type of inflammation by liberating arachidonic acid from phospholipids in the affected tissue. [34]. The production of eicosanoids from arachidonic acid occurs through three primary pathways. The cyclooxygenase pathway is responsible for the

synthesis of all eicosanoids with ring structures, such as prostacyclins, thromboxanes, and prostaglandins. The lipo-oxygenase pathway is utilized to produce hydroxylated derivatives of straight-chain fatty acids, namely leucotrienes, HETE (hydroxy eicosatetraenoic acids), and HPETE (hydroperoxy eicosatetraenoic acids). Reports indicate that the prostaglandins released, specifically, prostacyclines (PGI_2) and prostaglandin-E, can stimulate the A-fibers and result in the perception of pain. When the A-fibers are active, one may experience a sharp, localized pain [35]. Through the evaluation of the writhing effect caused by acetic acid injection and the ability of the test samples to prevent this effect, the analgesic activity was assessed. Any medication that decreases the number of writhing episodes demonstrates analgesic effects by inhibiting the production of prostaglandins, which is a mechanism that helps alleviate peripheral pain. This theory aligns with the hypotheses of certain researchers who suggest that the acetic acid-induced writhing test can be a useful tool for evaluating analgesics that have both peripheral and central effects [36]. When administered in higher doses, the MELA was found to decrease the occurrence of acetic acid-induced writhing in mice. A positive control was used, employing diclofenac sodium to inhibit the production of prostaglandins. By inhibiting the production and release of prostaglandins, it reduces the discomfort associated with arthritis, swelling, and inflammation [37]. The medication has an impact on the activity of polymorphonuclear leukocytes in vitro. It reduces chemotaxis, and the production of harmful oxygen-derived free radicals, superoxide radicals, and neutral proteases [38]. In animal experiment models, studies have demonstrated that diclofenac can effectively reduce inflammation caused by various phlogistic agents [35]. Considering the antinociceptive effects observed in the methanol extract of *Ludwigia adscendens* during various tests, such as the hot-plate and acetic acid-induced writhing tests, it is likely that this extract has both central and peripheral antinociceptive properties. This is supported by the similar responses seen in the positive control diclofenac.Formalin induces pain in mouse paws through two distinct pathways. Initially, the immediate effects of formalin injection result in the early phase, which is marked by neurogenic pain. This pain is triggered by the direct stimulation of sensory afferent fibers and the activation of C-fibers. During this phase, the induction of nociception also involves bradykinin and substance P. Second, during the late phase (15 minutes after formalin injection), various inflammatory mediators such as histamine, prostaglandins (PGs), bradykinin, serotonin, and others come into play in peripheral tissues, leading to the sensation of pain caused by inflammation[39]. The functional changes in the dorsal horn of the spinal cord area are another factor that contributes to the development of formalin-induced late-phase pain [58]. Based on the results of the formalin-induced paw-licking test, diclofenac, and MELA effectively reduced both stages of nociception. The strength of the inhibition intensified during the later phase and was dependent on the dosage. Peripheral analgesics, such as aspirin and hydrocortisone, primarily reduce the later phase of formalin-induced paw-licking in mice. On the other hand, central analgesics like opioids are capable of suppressing both stages [40]. The results of the hot plate test are backed by the significant reduction in paw lickings during the formalin test, indicating the potential pain-relieving effects of MELA. In addition, the

paw-licking deterrent during the later phase indicates the inhibition of inflammatory mediators, similar to what was observed in the acetic acid-induced writhing test. The anti-inflammatory and wound-healing properties of *Ludwigia adscendens*methanol extract may be due to the presence of flavonoids or flavonoid glycosides.

There was a slight toxicity observed in the toxicological test for MELA. Based on the phytochemical screening, it was discovered that MELA contained a notable number of alkaloids. Therefore, the chemical composition and levels of aspidosperma-type alkaloids may have an impact on the toxicological properties of MELA[41].

6. CONCLUSION

This research demonstrated that *Ludwigia adscendens*possess significant pharmacological effect due to presence of some rich phytoconstituents. In comparison to diclofenac sodium, the extract demonstrates a powerful antinociceptive effect. The anticancer effect is also moderate. It possesses a notable antidiabetic effect. Based on the acute toxicological activity test, it has been demonstrated that this plant can have a mild toxicological effect on animals when administered in higher doses. Researchers often employ a range of analytical techniques, such as GC-MS analysis, column chromatography, NMR, and in-vivo tests, to validate their results

7.ETHICAL APPROVAL AND CONSENT

This research adhered to the regulations established by the US Food and Drug Administration, the Declaration of Helsinki, and the International Conference on Harmonization. The Faculty of Science at Stamford University Bangladesh carefully reviewed and approved the research procedure and written consent form (reference number: SUB/ERC/202301). Every participant in the study was required to provide a documented consent form, and they were granted the freedom to withdraw at any given time.

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