

STUDY OF CHEMOPREVENTIVE ROLE OF PADDY HUSK ON CERVICAL ADENOCARCINOMA HUMAN CELL LINE (HELA CELLS)SHAYLA KABIR^{1*}, SARDER MOHAMMAD SHAHRIAR JAHAN², SAZIA NOWSHIN³,
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ABSTRACT

Objective: Cervical cancer is the fourth most prevalent cancer type and the fourth primary cause of cancer-related death among women worldwide. The deficiencies of current treatments, including severe side effects and the inability to prevent progression to the metastatic stage, necessitate the investigation of alternative agents.

Methods: The chemopreventive approach employing natural products such as Paddy Husk is acquiring considerable traction in the scientific community. This study examined the chemopreventive effects of Paddy Husk on HeLa cervical cancer cells. Using the TBEA method, the IC₅₀ of the husk was determined. To evaluate the antiproliferative activity with prolonged treatment exposure, HeLa cells treated with the IC₅₀ value were incubated for 8 days.

Results: The results demonstrated that Paddy Husk extract effectively inhibited the proliferation of HeLa cells throughout the duration of the treatment. Examination under the microscope revealed that Paddy Husk extract induces apoptotic characteristics, including cell contraction, membrane rounding, membrane blebbing, the formation of apoptotic bodies, and vacuolation. A mouse skin fibroblast cell line (L929) was used to assess the *in vitro* safety of paddy refuse extracts at various concentrations, revealing no toxic effects on normal L929 cells.

Conclusion: These findings are essential for advancing our knowledge and recognizing the potential function of Paddy Husk compounds in cervical cancer chemoprevention.

Keywords: Chemoprevention, Anti-proliferative, Vacuolization, Apoptotic body, Metastasis.

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INTRODUCTION

Cervical cancer, which originates in the cells of the cervix in the lower part of the uterus, is a major global health issue that kills 265,672 people annually and accounts for about 527,624 new cases, according to latest figures [1]. The disease has the lowest incidence rates in Western Asia and the highest in Eastern Africa, which includes Zimbabwe. Among women in low-and middle-income countries, like Nepal, cervical cancer is the second most common malignancy in the region of Southeast Asia and is a major cause of cancer-related fatalities. According to estimates from the International Agency for Research on Cancer (2012), Nepal's age-standardized incidence and death rates for cervical cancer are 19.0 and 12.0/100,000, respectively [2,3]. This information highlights the significant burden of cervical cancer, especially in developing nations, and calls for increased awareness and action.

The stage of cervical cancer at diagnosis determines the initial treatment: Surgery or chemoradiation. Patients may develop urinary and sexual problems, reducing quality of life [4]. Health-care practitioners must understand patient preferences for treatment side effects to provide individualized care [5]. Chemoprevention has been established to stop cervical neoplasia from progressing to invasive cancer stages. Chemoprevention involves chronically administering synthetic, natural, or biological chemicals to postpone or prevent cancer. Chemopreventive drug development has shifted towards intensive preclinical mechanistic investigation and biomarker identification before clinical trials throughout the past decade [6].

There is substantial evidence that plant-based diets help prevent cancer. Singletary (2000) defines cancer chemoprevention as pharmacologic therapies with synthetic or natural substances to prevent, reverse, or delay invasive cancer. Paddy Husk, a rice milling waste, contains phenolic chemicals and has biological activity [7]. Paddy Husk inhibits inflammatory cytokines and has anticancer effect on numerous cancer cells, according to multiple research [8]. This study examines apoptosis and cell cycle in HeLa cell lines to determine paddy husk's potential as a cervical cancer chemopreventive.

METHODS

The extraction and cell culture reagents were obtained from reliable vendors, with Gibco products being the primary source for the latter. Using a DIONEX ASE350 model, the accelerated solvent extraction method was used to thoroughly wash, dry, grind, and extract Paddy Husk. To be used later, the extracts were extracted, concentrated, and kept at -20°C. The cell culture technique followed the instructions on the ATCC data sheet, making adjustments as necessary. The ATCC provided HeLa cells, a cervical cancer cell line, which were then kept under particular circumstances to provide a regulated growth environment. To assess the cytotoxicity of Paddy Husk extracts, L929 cells - a mouse skin fibroblast cell line - were also employed and kept under comparable circumstances. Regular medium replacements were performed and cell confluency was tracked. Cells were subcultured once they reached 80-90% confluency. The procedure comprised centrifuging, washing, detaching, neutralizing, and seeding into fresh flasks. This methodical process guaranteed the cells' uniform

distribution and ideal growth. To promote proliferation, cells were then cultured under specific conditions. Paddy Husk extracts' potential as a cancer treatment was thus investigated in this study by combining exacting extraction procedures with intricate cell culture techniques.

Determination of inhibitory concentration (IC₅₀)

Using the formula $M1V1=M2V2$, the extraction of Paddy Husk was carefully prepared for the experiment and calculated for different concentrations. HeLa and L929 cells were then treated. HeLa and L929 cells were cultivated and kept at a particular temperature and humidity level until they reached 80–90% confluency. To observe the effects, the cells were subjected to subculturing and treated with several concentrations of Paddy Husk extracts. The inhibitory concentration (IC₅₀), which stops 50% of cell growth, was the focus of particular attention.

Under varied doses of Paddy Husk extracts, the HeLa cells showed morphological changes and characteristics associated with apoptosis, as detected at different intervals and magnifications using an inverted microscope. HeLa cells treated with the IC₅₀ of Paddy Husk extracts underwent a cell proliferation experiment; the viability of the cells was computed and plotted against time. The L929 cells were subjected to an IC₅₀ dose of Paddy Husk extracts, and their cell viability was plotted and analyzed using similar protocols.

In addition, L929 cells were subjected to the same cytotoxicity test settings, and observations were made about cell survival in response to varying concentrations of Paddy Husk. HeLa cell morphological alterations were detected by microscopic inspections between treated and untreated samples. Apoptosis indicators were found in these observations, which were captured on camera at 24, 48, and 72-h intervals using different magnifications.

The formula $\text{Cell viability (\%)} = \frac{N_v}{N_t} \times 100\%$ was utilized to determine the viability of HeLa and L929 cells. N_v stands for the total number of viable cells, while N_t represents the total cell population. To ensure precision and dependability in the observations and outcomes, the experiments were carried out in triplicate. Three separate trials were carried out for every set of studies, and the standard deviation (SD) was used to describe the findings. Statistical analyses were meticulously performed using SPSS 15.0 for Windows. The Independent Student's t-test and one-way analysis of variance (ANOVA) were utilized to evaluate significant differences between treated and untreated samples. Levels of significance were set at $p \leq 0.05$, $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.01$, and $p \leq 0.001$, $p \leq 0.001$, indicating statistical significance.

RESULTS

IC₅₀ of Paddy Husk waste on HeLa cell

The goal of the study was to find the IC₅₀ of tocotrienol, or Paddy Husk, on the human cervical adenocarcinoma cell line, or HeLa cell line. Using a range of doses of Paddy Husk (tocotrienol), the suppression of cell growth was monitored for a full 72 h. 0.03 mg/mL, 0.05 mg/mL, 0.07 mg/mL, 0.09 mg/mL, and 0.11 mg/mL were the doses used. It is noteworthy that the chemical exhibited a 32.00±1% inhibition of cell viability even at the lowest tested concentration. As the concentration reached 0.11 mg/mL, viability dropped to 32.83±1% as well. There was a clear negative association found between the content of the chemical tocotrienol from Paddy Husk and the viability of the cells. Using the manual measurement on the graph and the findings, the IC₅₀ value was found to be 0.07 mg/mL. The experiment was carefully carried out in three copies, and the (±SD) of the data was provided. This result demonstrates the possible inhibitory effect of tocotrienol (Paddy Husk) on HeLa cells.

Observation on the morphological changes of HeLa cells

When Paddy Husk (Tocotrienol) compound was applied to HeLa cells, it caused observable morphological changes as well as cell death (Fig. 1). When compared to the untreated cell group, direct observation with an inverted light microscope showed a decrease in the number of cells in the treated group along with various distinct morphological changes. Treatment-exposed cells rounded up, showed blebbing of the

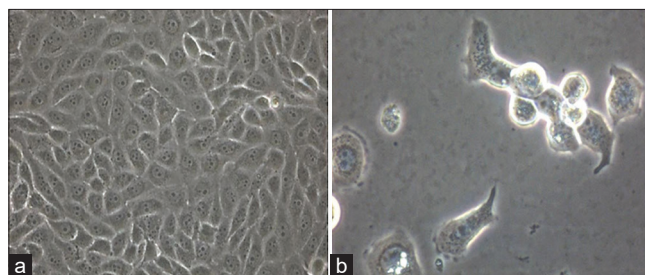


Fig. 1: (a) Showing healthy, full confluent HeLa cells before treatment. (b) Light micrograph of HeLa cells with 0.07 mg/mL Paddy Husk (Tocotrienol) compound treatment for 72 h. Some of the morphological changes, as shown in this figure, such as vacuolization, cell elongation, cell shrinkage, and rounding of cells. Pictures were in ×200 magnification

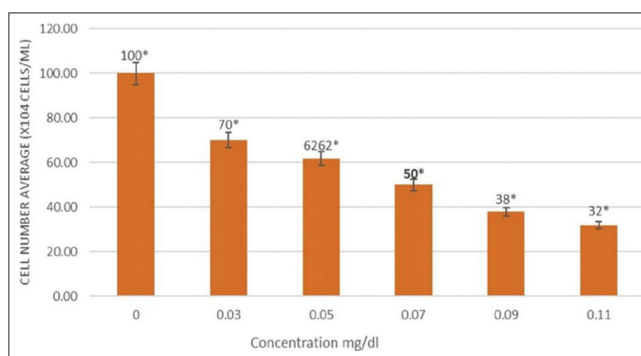


Fig. 2: IC₅₀ of HeLa cells treated with Paddy Husk (Tocotrienol) compound. Values were represented in ±SD manner of the triplicates with significant differences. **indicates (p<0.05) with respect to untreated cells by One-way ANOVA

membrane, and finally detached from the surface of the culture flask. When compared to the HeLa cells that had not been treated, these traits were clearly unique.

Anti-proliferation effect of paddy husk (Tocotrienol) compound on HeLa cells

HeLa cells treated to the IC₅₀ dose of Paddy Husk (Tocotrienol) compound, 0.07 mg/mL, for 8 days showed morphological changes and cell death. The inverted light microscope showed fewer cells than the untreated sample. Untreated HeLa cells proliferated, grew, and maintained their structure. The treated cells with the IC₅₀ dose of Paddy Husk (Tocotrienol) compound and the untreated control were compared over 8 days to determine HeLa cell viability in Fig. 2. First, 3rd, 5th, 6th, and 8th days showed significant cell viability decline. Paddy Husk (Tocotrienol) compound increased cell death, causing HeLa cells to vacuolate, shrivel, and elongate. On the 8th day of the experiment, the proportion of viable cells was 16±0.99%. Paddy Husk (Tocotrienol) inhibits HeLa cell development, according to these data.

Apoptotic effect of Paddy Husk (Tocotrienol) compound on HeLa cells

The inhibitory effect was further supported by a flow cytometry-based investigation of apoptosis. Following a 72-h incubation period, the apoptotic rates of the cells under examination showed significant variations. The percentages of apoptotic cells in treated and untreated HeLa cell populations are summarized in Table 1, which is based on data compiled from three different trials. The quadrant at the upper right location (Q2), which has high PI and Annexin V staining, indicates necrosis or late apoptosis. On the other hand, the right lower quadrant (Q4), which is primarily stained for Annexin V, denotes early cell apoptosis, while the upper left quadrant (Q1), which has significant PI staining, represents debris (Fig. 3). It is clear that treatment with the

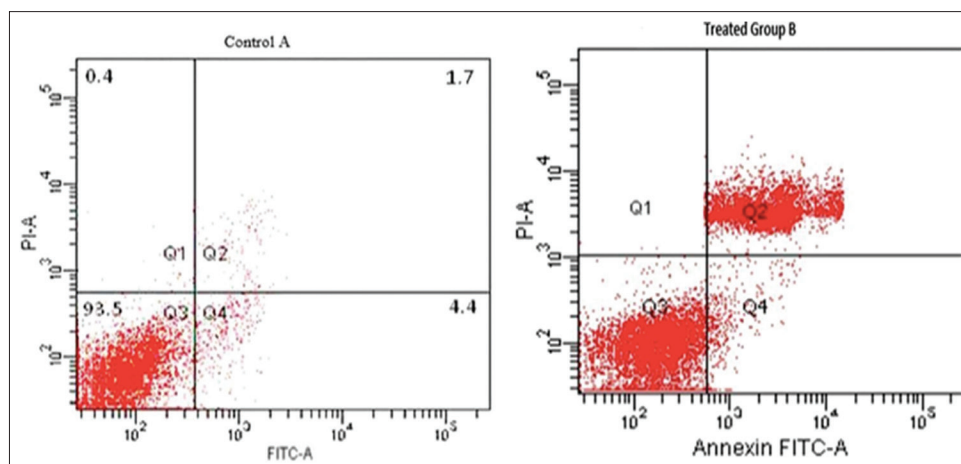


Fig. 3: Annexin V expression with 0.07 mg/mL Paddy Husk compound corresponded to untreated control cells for 72 h of incubation. FITC-A (horizontal) and PI-A (vertical) showed Annexin V and PI stains intensity, respectively. Q1: Dead cells/debris, Q2: Late apoptotic/necrotic cells, Q3: Live cells, Q4: Apoptotic cells

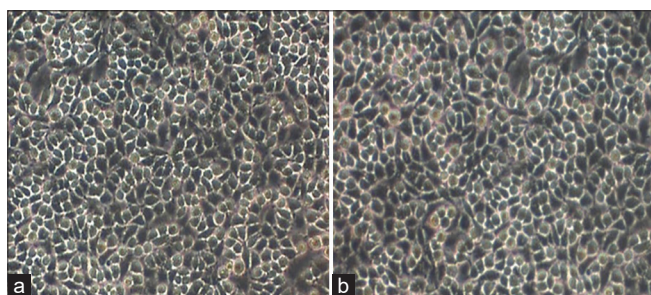


Fig. 4: (a and b) Light micrograph of L929 cells with 0.07 mg/mL Paddy Husk (Tocotrienol) compound corresponded to untreated control cells for 72 h of incubation. The picture was in $\times 100$ magnification

Paddy Husk (tocotrienol) chemical considerably promotes apoptosis in HeLa cells, given the observed relevance in the data.

Cytotoxicity test of Paddy Husk (Tocotrienol) compound on L929 cells

Normal mouse skin fibroblast (L929) cells were used to perform the cytotoxicity assessment to provide a more comprehensive understanding of the toxicity level of the Paddy Husk (Tocotrienol) compound on HeLa cells. The cytotoxic assay of the substance Paddy Husk (tocotrienol) on HeLa cells is shown in Fig. 4a, with a little decrease in the proportion of viable L929 cells. Comparing the cell viability results to the untreated cells, which showed a cell vitality of 95%, the normal cells showed no discernible cytotoxicity. Thus, it can be inferred that the concentration used was not very harmful to the L929 cell line. The morphological changes, as shown in Fig. 4b, matched the information. Under an inverted light microscope, observations were classified using Table 1 (ISO 10993-5, 2009).

DISCUSSION

Chemopreventive effects of paddy waste products

Cancer remains a significant health concern, and even with treatment options, incidence rates have not significantly decreased. Because of this, a great deal of study has been done to find effective treatments for different types of cancer, looking at both natural and artificial chemicals. The research presented here conducted *in vitro* studies with cultivated HeLa cells to identify putative indicators for chemoprevention. It is hypothesized that the elevation of bioactive chemicals such as phenolics, Vitamin E, γ -aminobutyric acid, γ -oryzanol, and phytic acid plays a role in mediating the anticancer action. The bioactivity of rice and its derivatives is still not fully understood, though [9].

Table 1: Shown the percentage distribution of cells stained with Annexin V and PI, after being exposed to a 0.07 mg/mL concentration of Paddy Husk (Tocotrienol) compound for 72 hours

Cells	Viability (%)	Early apoptosis (%)	Late apoptosis (%)	Total (%)
Untreated	77.12 \pm 1.01	16.96 \pm 0.80	5.6 \pm 0.60	22.56 \pm 1.4
Treated	63.14 \pm 2.13**	20.13 \pm 0.57**	8.5 \pm 0.70*	28.63 \pm 1.27

The percentage of apoptotic cells was determined by flow cytometry. The value represents means \pm SD of the triplicates. There was a significant difference between Paddy Husk (Tocotrienol) compound treated and untreated ($p > 0.05$)

Inhibitory and cytotoxic activities of paddy waste product on cervical cancer cell line (HeLa)

The Trypan blue exclusion assay shows that rice waste product extracts suppress HeLa cell growth *in vitro*. The compound's IC_{50} value of 0.07 mg/mL showed a significant growth inhibitory effect in treated HeLa cells compared to untreated ones. The IC_{50} was 0.01 mg/mL in a prior tongue cancer cell investigation; however, this value is greater. In the study, 0.07 mg/ml of Paddy Husk chemical inhibited rapid HeLa cell proliferation. Higher compound concentrations increased inhibition. Conventional cytotoxic treatment works better on fast-growing tumor cells [10]. Most cancer medicines are harmful to both malignant and healthy cells [11]. Natural chemicals are appealing as chemopreventive agents since they are less harmful [12]. Cytotoxicity tests are fast, sensitive, standardized, and inexpensive for finding physiologically hazardous components in materials [13].

Selecting the appropriate cell line for cytotoxicity testing is essential, as long as the mammalian cell lines are of a consistent enough quality to be repeated. Still, human or rodent cells are usually more useful, especially if more *in vivo* or *in vitro* research is planned [14]. To ensure that treatments with rice husk do not damage normal cells, the cytotoxicity of paddy extract on normal mouse skin cell lines (L929) was investigated. Furthermore, even at increasing extract dosages, the IC_{50} dose of paddy extracts, as evaluated on HeLa cells, showed no toxicity to L929 cells, highlighting the potential of paddy waste product extracts as promising chemopreventive agents with low cytotoxicity to normal cells. This study looked at the effectiveness of products made from paddy husks against the HeLa cell line, a cell type used to study cervical cancer.

The rice husk compound's antiproliferative capability was first evaluated by calculating the IC_{50} value at different concentrations tested on a cervical cancer cell line. When compared to their untreated

counterparts, the treated cervical cancer cells showed an increased growth inhibitory impact. The results showed a significant inhibitory effect on cervical cancer cells, with an IC_{50} value of 0.07 mg/mL determined. Still, this figure was more than what was found in a prior investigation [15]. The study found that the Paddy husk component, at a dosage of 0.07 mg/mL, significantly suppressed the proliferation of HeLa cell lines that were proliferating at a high rate. The chemical showed an enhanced inhibitory action at concentrations higher than 0.07 mg/mL in comparison to the lower doses. Fast-growing tumor cells are generally more amenable to standard cytotoxic chemotherapy than are slower-growing tumors that show resistance to these therapies [16].

Paddy waste product inhibits proliferation and induces apoptosis

Apoptosis may explain rice husk extracts' suppression of HeLa cell growth or death. A microscope was used to observe HeLa cell morphology before and after treatment. Flow cytometry showed that the extracts caused cervical cancer cells to undergo early apoptosis. The treatment did not significantly produce necrotic cells, excluding necrotic cell death. Paddy Husk extracts inhibited cellular growth and stimulated apoptosis in HeLa human cervical cancer cells at IC_{50} concentrations. HeLa cell proliferation was significantly inhibited by the cell proliferation assay, as measured by viable cell count. The rice husk extracts' strong anti-proliferative effect may be due to accelerated apoptosis, as seen by inverted phase-contrast microscopy, which shows cell shrinkage and membrane blistering. These findings support its use in detecting apoptosis [17,18]. Apoptosis research showed that Paddy Husk extracts induce apoptosis, suggesting that they may kill HeLa cells.

CONCLUSION

This study uses *in vitro* cell culture to show how Paddy Husk chemicals affect HeLa cells. Specifically, it showed HeLa cell susceptibility to Paddy Husk chemicals. These chemicals greatly decrease cell development and HeLa cell proliferation. Paddy Husk's propensity to trigger HeLa cell apoptosis suggests chemoprevention. Various Paddy Husk chemicals induce apoptosis in HeLa cells to restrict their proliferation with minimal harm on normal cell types. This, together with a sustained antiproliferative action and high triggered apoptosis, is notable. The capacity to limit cancer cell multiplication without harming normal cells is important.

We were unaware of any earlier research on rice husk chemicals and HeLa cells when this study was undertaken. These chemicals may offer a unique approach to cancer treatment, therefore studying their inhibitory potential is crucial. This study suggests that rice husk chemicals may treat cancer, specifically cervical cancer.

AUTHORS CONTRIBUTION

All the authors contributed equally to this study.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

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