Evaluation of Compound Hordenine (*Hordeum vulgare*): *In-vitro*, Gene Expression, and Molecular Modeling Investigations

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ABSTRACT:

This study aims to investigate the effects of a compound named hordenine on cells. We examined its impact on gene expression, inflammation, and cell proliferation. To do this, we took unique steps. We started by examining how effectively cells were developing. To gauge how quickly they were multiplying, we conducted a test. We also counted the number of cells by using a machine. Then, we performed a specialized test to evaluate the inflammatory marker IL-6. We also used a technique called RT-PCR to examine particular genes in the cells. Additionally, we used a computer simulation to examine how hordenine interacts with the specific protein MMP1. The findings demonstrated that even a modest amount of hordenine significantly aided cell growth. It also diminished. The stuff called hordenine helped lower inflammation more than another thing called rotenone. Cells treated with hordenine made less of something called IL-6. When we looked at certain genes in skin cells that were treated with hordenine, they seemed more active. Hordenine also binds well to a protein with an energy of -4.5 Kcal/mol. Because of all this, we think hordenine is good for cells. It helps cells grow and can triplicate when they're not working well.

KEYWORDS: Hordenine, Viability of Cell, Proliferation of cell, anti-inflammatory activity, studies on gene expression, molecular modeling.

INTRODUCTION:

Tertiary amines, such as the chemical hormone (hordenine), are related to N-dimethyltryptamine. It is a significant component of the Poaceae family of plants known as barley (Hordeum vulgare Linn). Anhaline, 4-[2-(Dimethylamino) ethyl] phenol, and 4-(2-(Dimethylamino) ethyl) phenol are some additional names for it. C10H15NO is the chemical recipe for it. Its form is similar to the energy-boosting compounds in bitter oranges.

Hordenine has a variety of uses, including the treatment of stomach disorders, lung problems, elevated prolactin hormone levels, diabetes and related conditions, weight loss, fitness, and even muscular health. It may also lessen pain, combat bacteria, and help fight off some infections. It is even capable of blocking some bodily impulses.

In addition, it functions as a marker in beer and can interact with the dopamine D2 receptor in the brain.[1]

The no. of healthy, live cells in the using sample is referred to as cell viability.[2] For building or tissue remodeling, the two important metrics are cell viability and cell proliferation. They will provide important reviews on the material's or structure's compatibility with surface alterations, three-dimensional structure, oxygen transport, compatibility with deteriorated products, and many other specifics.[3,4] The number of live cells is measured by cell viability, while the number of daughter cells produced by a cell population is measured by proliferation[5].

Interleukin (IL)-6 has a crucial role in controlling the remodeling and inflammation process. Processes involving interleukin include leukocyte activation, differentiation, and the development of fibroblasts, keratinocytes, and endothelial cells. One unique protein that can help reduce inflammation and also act as a calmer is IL-6. It is composed of a wide variety of cells. The particular cell, the cause of the production, and the genes of the cells producing the IL-6 all affect how much of it is produced[6,7,8,9].

Matrix metalloproteinases (MMPs) affect several basic physical processes like wound healing, tissue remodeling,

angiogenesis, and uterine and mammary involution, as well as significant functions like cell adhesion, migration, and proliferation. MMPs can appropriately regulate the extracellular and pericellular environment's homeostasis. Studies on gene expression frequently employ these[10,11,12]. MMPs, including MMP-8, MMP-9, and MMP-7, have defensive or anti-inflammatory properties. The cellular origins of various MMPs have been extensively researched[13].

MATERIALS AND METHODS:

Materials:

The drug hordenine and other chemicals used are procured from sigmaaldrich.com and preserved in ambient temperature

Cell viability evaluation by using MTT Assay:

MTT was used to assess the hordenine cell viability on keratinocytes. Using a proliferation MTT assay kit and following the instructions of the manufacturer, one can estimate the cell growth rate (Sigma Aldrich). In a nutshell, 3000 cells/well of keratinocytes are grown in plates with 96-well and kept for incubation for 24 hours at 37ºC with CO_2 of 5%. keratinocytes were cultured in conditions containing rotenone, hordenine, and control. After draining the liquid from the wells, 100µl of DMSO was applied to solubilize the formazan crystals that had adhered to the wells. Healthy cells form from these crystals. The amount of light absorbed by the solution at a 540 nm wavelength was measured using a device known as an ELISA plate reader, and at a 720 nm wavelength of reference utilized for comparison [14, 15].

Using the formula, the percentage of living cells at various extract concentrations was determined:

$$\% \text{ Viability of cell} = \frac{\text{Total no. of cells} - \text{Total no of dead cells}}{\text{Total no. of cells}} \times 100$$

Assay of cell cycle:

96-well plates were used to plate the keratinocyte cell lines. After that, these cells were exposed to either a chemical that had no effect (such as water) or varying concentrations of hordenine (20µg/mL) that had been combined with rotenone. To observe how this influenced the cells' growth and division, we employed a device known as flow cytometry. We used the equipment from Heidelberg, Germany-based Becton-Dickinson, and adhered to their instructions. The data showed the proportion of cells in each of the three development phases: G0/G1, S, and G2/M[16,17].

Interleukin 6 Inflammatory Marker Estimation:

Twelve-well plates were used to seed the keratinocytes, with one well holding one thousand cells and being incubated at 37°C. To induce inflammation, 1µg/ml of lipopolysaccharide was given to each well when the cells had reached 80% confluency. Following a 24-hour incubation period, the medium was removed, cleaned with phosphate-buffered saline, and fresh media was added. Various drug concentrations were then added. Following incubation, the ELISA protocol was used[18,19].

Studies on Gene expression of MMP1 and MMP9:

RT-PCR in Quantification:

For about 24 to 36 hours, keratinocyte cells were treated with DMSO, a precisely measured quantity of hordenine, or comparable compounds. Using a kit from RNASure small isolation kit Nucleo-pore, Genetix, we extracted the genetic material (RNA) from these cells. Next, we used the cDNA synthesis kit from Thermo Fisher with a specific starter and the instructions from Emerald Amp® GT PCR Master Mix (Takara) to create a type of genetic copy known as cDNA. Using distinct starting points for every gene, we examined the function of each gene (Table 1). The test procedure was as follows: 1) Heat the genes for 3 minutes at 95 °C; 2) Repeat 30 times at 95 °C for 1 minute; 3) Hold the genes for 30 seconds at 57 °C (depending on the starters used); 4) Heat at 72 °C for one minute; 5) Hold at 72 °C for seven minutes. We examined the melting patterns of the genes to verify the outcomes. To see how these genes altered, we compared the quantities of these genes[20,21,22,23].

Molecular modeling:

We utilized a unique script written in the Perl programming language (using the command "perl vina_windows.pl") to accomplish this. The protein that we examined was MMP1, which we obtained from PDB: 3SHI. To observe the data more clearly, we also used Biovia Discovery Studio 4.0 and PYMOL software. We concentrated on the vigor and solidity of the bonds. Based on the strength of the link, which we quantified using parameters like binding energy (measured in kcal/mol) and dissociation constant (measured in pM), we condensed the results. A stronger connection was indicated when the energy was more negative[24,25,26].

RESULTS:

Cell viability assay: In our lab research, we treated keratinocytes (5, 10, 20, and 30µg/ml) with rotenone and hordenine for 24 hours. The vitality of the cells is assessed 24 hours after the exposure point. Every experiment should be conducted twice, with each time point being recorded in duplicate wells. When compared to other substances, there is a low concentration of hordenine (5µg/ml) and a high percentage of cell viability (97%). [27, 28].

Table 1: Cell viability %

Drug (µg/ml)	% of Cell Viability
Control	99.6
Rotenone (5)	83.4

Rotenone (10)	81.3
Rotenone (20)	76
Rotenone (30)	75.7
Hordenine (5)	98
Hordenine (10)	93.8
Hordenine (20)	88.6
Hordenine (30)	83.6

Fig. 1: Using the MTT assay, determine the percentage of viable keratinocytes following a 24-hour incubation period supplemented with hordenine, and rotenone.

Assay of cell proliferation:

Hordenine and rotenone were applied to keratinocytes for 36 hours. As a baseline, untreated cells were employed. We examined the portion of cells in various growth phases of the cycle after 36 hours. The findings demonstrated that, in comparison to the control and other treatment groups, the hordenine-treated cells had more cells developing. When treated with hordenine, a sizable fraction of keratinocytes grew in the G0/G1 phase, according to the growth cycle analysis. On the other hand, a sizable portion of the keratinocyte cell line's cells was discovered in both the G0/G1 and S phases after receiving the same therapy. This implies that hordenine efficiently promotes cell development at different phases of the growth cycle.

Control

Hordenine

Rotenone

Fig 2: Cell proliferation at different phases

Evaluation of the Inflammatory Marker Interleukin-6:

The cells were treated with rotenone and hordenine after being stimulated with lipopolysaccharide and incubated for 24 hours. Comparing hordenine to rotenone, a substantial reduction in inflammation was seen. Hordenine-treated cells also secreted IL-6 at lower levels.

Table 3: Response of Inflammation.

	Standard	LPS	Rotenone	Hordenine
2000 (pg.ml)	1.955	1.31	0.765	0.168
1000 (pg/ml)	1.905	1.508	0.895	0.194
500(pg/ml)	1.44	1.344	0.765	0.302
250 (pg/ml)	1.24	1.056	0.557	0.139
125 (pg/ml)	1.1	0.9	0.8	0.2

Studies on gene expression: Three genes (MMP-1, MMP-9, and IL-6) were measured for expression levels in keratinocytes using real-time polymerase chain reaction (RT-PCR). There was a notable rise in the expression levels of the genes encoding matrix metalloproteinase MMP-1, MMP-9, and IL-6 in particular. The results of our study on the impact of hordenine on keratinocyte gene activity showed that the expression of these genes rose, suggesting that hordenine influences the activity of these genes.

MMP1

GADPH

MMP9

Studies of Molecular Docking
Hordenine's three-dimensional interaction image at MMP1's active location

Hordenine's two-dimensional interaction picture at MMP1's active location
Blue aromatic areas at the active site
Hydrophobic areas (gray) at the location of activity
At the active site, H-bond donors and acceptor regions

At the active site, solvent-accessible surface areas are shown in blue.
Hordenine - MMP1 intricate 3D picture

Drug	Distance	Bond	Type of Bond	Type of Amino acid
Hordenine	2.21883	Hydrogen	Conventional Hydrogen	TYR241
	3.74722	Hydrogen	Carbon Hydrogen	GLU218
	3.52514	Hydrogen	Carbon Hydrogen	ALA183
	4.09468	Hydrophobic	Pi-Alkyl	LEU182

4.99468	Hydrophobic	Pi-Alkyl	LEU182

The binding energy of hordenine is -4.5 Kcal/mol. Hordenine formed hydrophobic (Pi-Alkyl) bonds with leu 182, carbon-hydrogen bonds with glu 218 and ala183, and conventional H-bonds with tyr241 residue.

DISCUSSION:

This work has shown the ability of the compound hordenine to support the survival and growth of keratinocytes. Important cells in the skin's outer layer are called keratinocytes. They are distinct due to their size, interconnectedness, and abundance of cytoplasm. The outer skin's four layers includes these cells. (29) The viability of a cell is defined as no. of live cells and, typically, a comparison to the normal state. About 97% of the cells survived at 5µg/ml, the lowest concentration of hordenine. This exceeds the levels of other chemicals, such as rotenone [30].

When compared to the control and other groups, the hormone treatment promoted the proportion of cell proliferation. When treated with hordenine, a considerable number of keratinocyte cells expanded during the G0/G1 phase, according to an analysis of the cell growth stages. In the keratinocyte cell line, hordenine treatment resulted in a great proportion of cells in the G0/G1 and S phases. This demonstrates how hordenine efficiently promotes cell development at different phases of the growth cycle.

Interleukin-6 has a critical action in wound healing promptly and in the management of rapid inflammation[31, 32]. Inflammatory signals are released by keratinocytes, tissue-resident macrophages, stromal cells, and endothelial cells in response to IL-6. Furthermore, IL-6 will draw white blood cells to an area of the wound. The signals from IL-6 help change the environment in a way that promotes healing when inflammation increases [33, 34]. We used keratinocytes exposed to lipopolysaccharide to investigate hordenine's anti-inflammatory properties. When we examined the amount of IL-6, a chemical that indicates inflammation, we discovered that hordenine significantly reduced inflammation in comparison to rotenone. This demonstrates the anti-inflammatory benefits of hordenine.

We measured the amount of gene activity using real-time PCR. The amount of RNA was normalized by comparison with the GAPDH reference gene [35, 36]. We found that the expression of the MMP-1, MMP-9, and IL-6 genes had significantly increased. We found that these genes were more active in human keratinocytes treated with hordenine.

The process of predicting how a certain material (ligand) will fit into the structure of a known protein is called molecular docking. Using AD Vina scoring, the docking findings were examined in terms of binding energy and dissociation constant. A higher negative binding energy indicates a stronger relationship. The binding energy of hordenine was found to be -4.5 kcal/mol.

CONCLUSION:

The reports demonstrate the distinctiveness of the in-vitro work. We are the earliest to show that keratinocyte cell motility and proliferation may be enhanced in a lab setting by varying the quantity of hordenine (5, 10, 20, and 30µg). Hordenine appears to support cell viability, proliferation, and the establishment of a favorable environment for healthy growth—all of which may help with a variety of problems. We saw the same impact in our keratinocyte model. Our results highlight hordenine's potential as a useful compound with a range of actions, indicating potential medical uses. Additional testing in clinical settings and on living creatures is required to develop and validate new medicinal compositions utilizing hordenine.

CONFLICT OF INTEREST:

Here no disagreements of interest among the authors related to this research.

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