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RESEARCH ARTICLE

DETERMINATION OF THE ANTIPROLIFERATIVE EFFECT OF *STERNBERGIA LUTEA* (L.) KER GAWL. EX SPRENG. EXTRACTS ON A375 MALIGNANT MELONOMA CELL LINE

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Abstract

Epidemiological evidence confirms that plants are primary sources of drugs used to reduce the incidence of cancer and prevent cancer-related deaths. *Sternbergia* species are used for therapeutic purposes due to the amaryllidaceae alkaloids, lectins, phenolic acids, pigments, and volatile components they contain. In this study, the anticancer properties of *S. lutea* extracts were tested on the A375 malignant melonoma cell line. In addition, in the study, the transcriptional expression of *BCL-XL* and *Cas9* genes, which function in cell proliferation and apoptotic pathways, in cells treated with plant extracts were determined by qRT-PCR. According to the cytotoxicity results made by the MTT test, the highest inhibition percentage was determined at the plant's concentration of 500 µg/*m*L. At this concentration, A375 cells were inhibited by 83.63%, and the IC_{50} value of the extract was calculated as 194.64 µg/*m*L. In addition, in qRT-PCR analyses, a statistically significant increase was observed in the mRNA expression levels of Cas9 genes, which are positively correlated with the apoptotic pathway, in the extract and cisplatin-applied groups compared to the control group.

Keywords

Anticancer,
Antiproliferation,
BCL-XL,
Cas9,
Cisplatin

Time Scale of Article

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1 . INTRODUCTION

Researching the effects of medicinal plants on health is important for the discovery or design of new drugs. Plants will continue to be the best source for the production of medicines used to treat different diseases in the past, today and in the future [1-2]. Although there are many synthetic drugs designed from raw materials obtained from plant isolates, the diversity of diseases that people are exposed to and

the fact that people respond differently to diseases increase the importance of drug studies. It is estimated that acceptable therapy is available for only one-third of known human diseases. Therefore, revealing the biological characteristics of medically important species is important for future studies [3-5].

Plants' active ingredients offer antioxidant qualities that help the body combat dangerous free radicals, which are the root cause of many diseases [6-9]. The genus *Sternbergia* is significant among geophytes because it produces a class of naturally occurring antioxidants. The *Sternbergia* is a genus of bulbous monocotyledonous plants in the family Amaryllidaceae. The alkaloids that plants in the Amaryllidace family generate are highly valued in addition to their aesthetic qualities. The genus *Sternbergia* is one of the sources of many alkaloids. Several of these alkaloids have intriguing biological and/or pharmacological characteristics [10-11].

In phytochemical studies on *Sternbergia* species, Amarylidaceae alkaloids, lectins and phenolic acids were obtained. In addition, pigments and volatile components have also been investigated. Among the alkaloids isolated from Sternbergia species, one of the most important ones in terms of treatment is the alkaloid named galantamine. This compound is a competitive cholinesterase inhibitor with long-lasting central action and is used in the treatment of cholinergic-related neurodegenerative diseases such as Alzheimer's disease. Another interesting Amaryllidaceae alkaloid isolated from Sternbergia species is lycorine. Lycorin is antiviral against some RNA and DNA viruses. There are also studies on the interaction of lycorin with DNA and/or RNA and its antitumour activity by using different analysis methods [12-15].

In this context, in the present study, the antiproliferative properties of the plant extracts were investigated in A375 Malignant Melonoma cell lines using *S. lutea* (L.) Ker Gawl. ex Spreng. (Figure 1) extracts. In addition, the expression of *BCL-XL* and *Cas9* genes, which are thought to be involved in cell proliferation and apoptotic pathways, at the transcription level were also examined in the extracted cell lines. This study was produced within the scope of Zemheri Şaman's master's thesis.

Figure 1. Natural population of *S. lutea* (L.) Ker Gawl. ex Spreng (Muğla, Türkiye).

2. MATERIAL and METHODS

2.1. Plant Material

The *S. lutea* natural samples [16-17] were collected in the Menteşe district of Muğla (Türkiye). The bulbs of the collected plants were dried in a dark room under airflow conditions at room temperature (Figure 2).

2.2. Plant Extraction

Dried *S. lutea* samples were physically ground using liquid nitrogen. 10 g of powdered plant material was transferred into a flask and 40 ml of ethanol (96%) was added. It was then extracted in an ultrasonic water bath at 25°C for 30 min at 100% vibration. After extraction, the mixture was centrifuged at 4000 rpm for 5 min and the supernatant was removed. The same procedure was repeated by adding 40 ml ethanol to the pellet. The supernatants obtained from both processes were filtered using Whatman filter paper and the particles were removed. After transferring the supernatant to the beaker, ethanol was removed and the extract obtained was stored at +4°C [18-20].

2.3. Determination of Cytotoxic Activity

2.3.1. Passaged cell cultures

A375 Malignant Melonoma cell line was cultured in 25-well flasks containing DMEM medium containing 10% fetal bovine serum (FBS) and 1% antibiotic. Cells that reached sufficient growth were removed with Trypsin-EDTA, counted with trypan blue and then transferred to 96-well microplates and incubated [3].

Figure 2. *S. lutea* bulb samples used in the study.

2.3.2 Determination of cytotoxic dose

The cytotoxicity of *S. lutea* extract on A375 cell lines was determined by MTT method [21]. Cells were transferred to 96-well plates containing 10000 cells per well and incubated at 37°C for 24 h with 5% CO_2. Cells treated with serial dilutions of the extract were incubated in 5% CO_2 at 37°C for 24 hours.

After 24 hours, 20 µl MTT was applied to the wells and incubated for 3 hours. After incubation, 100 µl dimethyl sulfoxide (DMSO) was applied to the wells and measured at 540 nm in a spectrophotometer kept at room temperature in a shaking incubator for 20 minutes. Test samples were used in the concentration range of 15.625-500 µg/ml. IC_{50} value was calculated statistically [21]. The cisplatin was used as a control to compare the antiproliferative activity of the plant extract.
(%) Vitality = [100 × ($Sample_{abs}$) / ($Control_{abs}$)]
$Sample_{abs}$: Absorbance in wells treated with test material
$Control_{abs}$: Absorbance of the control well
(% inhibition) = 100 - (% viability)
IC_{50} value of *S. lutea* extract was calculated statistically [21].

2.4 Molecular Analyses - Determination of Gene Expression at Transcriptomic Level

RNA was extracted from A375 cell line using Thermo Scientific™ Gene JET RNA Purification Kit (Cat.No. K0732). For cDNA synthesis, total RNAs were reverse transcribed using OneScript® Plus cDNA Synthesis Kit (Cat.No. G236) and oligo-dT primers included in this kit. Amplification of the reverse transcribed RNAs was determined by Real-Time PCR using Ampliqon RealQ Plus 2 × Master Mix Green Kit (Cat. No. A323402) and in the presence of primers for the two genes of interest. The respective genes and the sequences of their forward and reverse primers are given in Table 1. The thermal cycle used in the reaction was denaturation at 95 °C for 30 seconds followed by binding at 55-58 °C for 30 seconds and elongation at 72 °C for 30 seconds. Real-Time PCR was repeated six times and analysed. All groups were analysed for the expression of *β-actin*, a housekeeping gene, and interpreted using the $2^{-\Delta\Delta Ct}$ [2^(-delta delta Ct)] method [22]. The mean of the obtained values (with standard deviation and standard errors) was calculated and a graph was created. The significance of the expression levels of the two target genes obtained after qRT-PCR compared to the control group was statistically analysed by T-Test method. T-Test data with $p \leq 0.05$ were considered statistically significant [23-24].

Table 1. Primers and sequences designed for use in Real-Time PCR [25].

Gene	Primer sequence
β-actin	F: 5’ TCCTCCTGAGCGCAAGTACTC 3’ R: 5’ CTGCTTGCTGATCCACATCTG 3’
BCL-XL	F: 5’ GCTAGCAGACTTTGGACTAGCCAG 3’ R: 5’ AGCTCGGTACCACAGGGTCA 3’
Cas9	F: 5’ GGCTGTCTACGGCACAGATGGA 3’ R: 5’ CTGGCTCGGGGTTACTGCCAG 3’

3. RESULTS

3.1. Anticancer Properties of *S.lutea* Plant Extract

In this study, the cytotoxic activity of the rhizome extract of *S. lutea* on A375 melanoma cells *in vitro* was determined by MTT assay. The highest inhibition among the test concentrations was determined at a concentration of 500 µg/*m*l. At this concentration, A375 cells were inhibited by 83.63%. At the lowest concentration, 20.85% inhibition was observed (Figure 3). IC_{50} value of the extract was calculated as 194.64 µg/*m*l. In order to compare the anti-proliferative activity of the plant extract, A375 cell line was treated with cisplatin as control. Cisplatin was applied at concentrations between 1.6 and 50 µg/ml. The highest inhibition was 85.82% at 50 µg/ml and the lowest inhibition was 10.72% at 1.6 µg/ml (Figure 4).

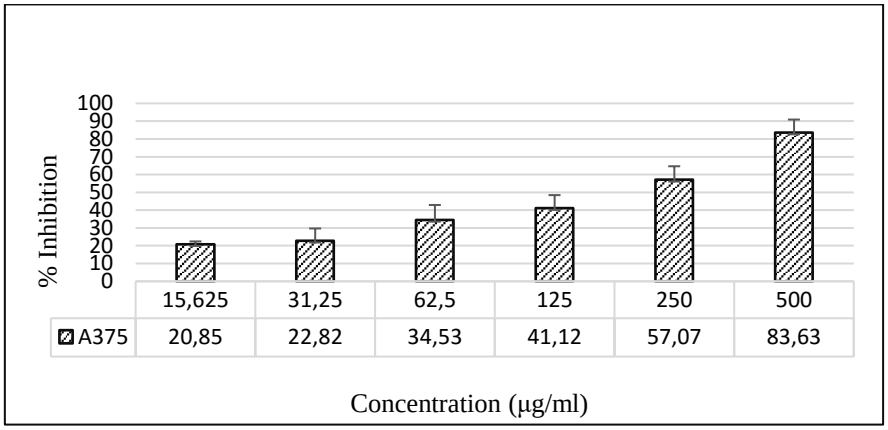

Figure 3. % inhibition values determined by MTT test in A375 cell line treated with *S. lutea* extract.

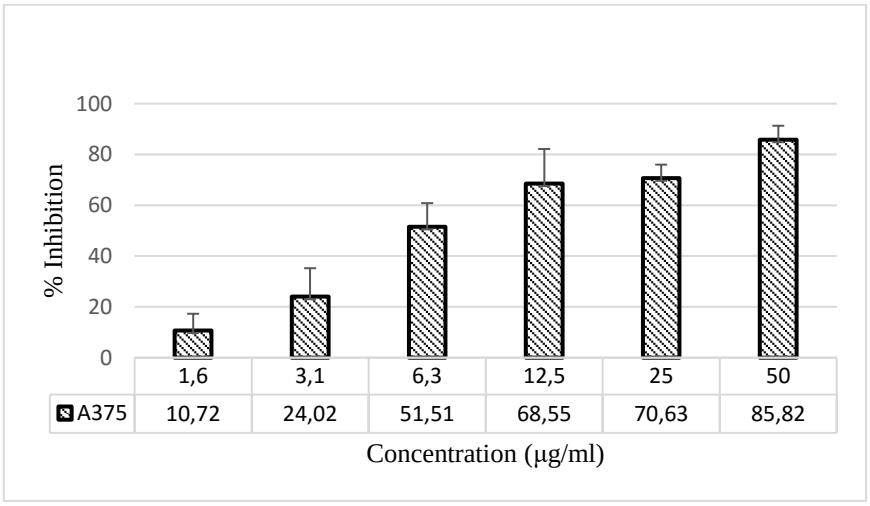

Figure 4. % inhibition values determined by MTT assay in cisplatin-treated A375 cell line.

3.2. Transcriptomic Analysis in A375 Cell Line

The mean of the Ct values (with standard error and standard deviations) obtained after Real-Time PCR to determine the expression levels of *BCL-XL* and *Cas9* genes at mRNA level in A375 cell line were calculated and graphs were created. When the data of A375 cell line were analysed, a decrease was observed in the mRNA expression level of *BCL-XL* gene, which is negatively correlated with the apoptotic pathway, in the extract and cisplatin groups, respectively, compared to the control group. However, this increase was not statistically remarkable. A statistically significant increase was observed in the mRNA expression levels of *Cas9* genes, which are positively correlated with apoptotic pathway, in the extract and cisplatin treated groups compared to the control group. It is a remarkable result in this study that treatment with S. lutea plant extract for 6 hours induced the expression of *Cas9* gene more than cisplatin treatment used as an anti-cancer drug and caused serious side effects (Figure 5).

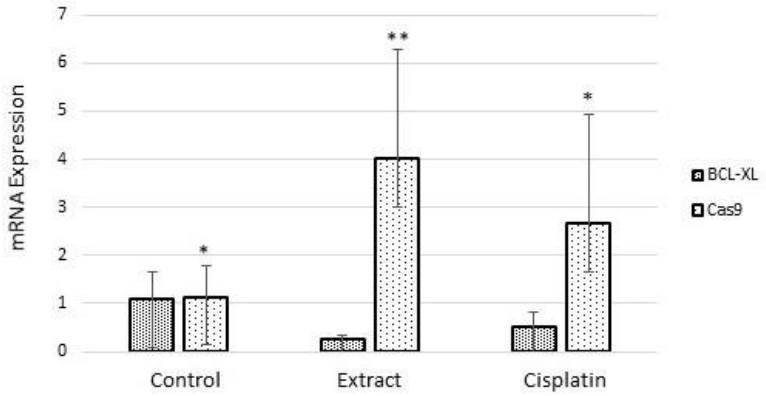

Figure 5. Relative mRNA expression graph of *BCL-XL* and *Cas9* genes in A375 cell line (Control: No substance treatment; Extract: Treated with 192.64 µg/ml *S.lutea* plant extract for 6 h; Cisplatin: Anti-cancer treatment; treated with chemotherapeutic drug at a concentration of 7.87 µg/ml for 6 h).

4. DISCUSSION and CONCLUSION

The cytotoxic effect of the extract obtained from the rhizome parts of *S. lutea* was investigated on A375 Human Malignant Melanoma cells. Inhibition was found to increase depending on the dose. This type of cancer, which was rare in the past, has become a more common cancer type day by day [26-27]. The cause of 75% of deaths due to skin cancer is melanoma cancer [28]. The Amaryllidacea family, including S. lutea, is known to contain many alkaloids. One of them is hypamine and it is a type of alkaloid that can be obtained from *S. lutea* [29]. Another type of alkaloid, lycorine, has been found to have anti-proliferative effect as a result of studies [30]. S. lutea contains many other alkaloids [31]. In a study by Masi et al. [32], inhibition values were investigated using MTT method on different alkaloids. In this study, SK-MEL-3 cell line, a different type of skin cancer, was used and the presence of anti-cancer activity was determined as a result of the study. IC_{50} value was given as >50 µM.

In this study, the IC_{50} value of the ethonolic extract of S. lutea on A375 cells was calculated as 194.64 µg/mL. The difference in IC_{50} values between the two studies is thought to be due to the difference in cell lines. The cytotoxicity of the ethanolic extract of *S.luta*, whose IC_{50} value was determined in this study, on cancer cells can be investigated in future studies.

As a result of this study, it was observed that the % inhibition rates of A375 Human Malignant Melanoma cells treated with *S. lutea* extract *in vitro* for 24 hours increased depending on the dose increase. In the light of the data obtained from this study, it will be possible to investigate the effect of *S. lutea* plant, which has cytotoxic effect on melanoma cells, on other cancer cells and to create pioneering data for new drug searches.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

CRediT AUTHOR STATEMENT

Zemheri Şaman: Investigation, Formal analysis, Writing - original draft, Visualization**, Sevil Yeniocak:** Investigation, Formal analysis, Visualization**, İrem Demir:** Investigation, Formal analysis, Visualization**, Ergun Kaya:** Conceptualization, Supervision, Investigation, Formal analysis, Writing - original draft, **Nurdan Saraç:** Conceptualization Investigation, Formal analysis, Writing - original draft.

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