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Exploring the Therapeutic Efficacy of Iraqi Frog Bile Extract on SKGT4 and AMGM Cell Line Activities

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Abstract

The potential of natural extracts in cancer treatment has garnered significant attention, particularly for their ability to target cancer cell proliferation. This study investigated the cytotoxic effects of bile juice extracted from the Iraqi Eastern Green Frog on two cancer cell lines: SK-GT-4 (esophageal cancer) and AMGM (glioblastoma). This research showed that Iraqi frog bile juice can kill cancer cells of the SK-GT-4 and AMGM types, but only at certain doses. The MTT assay results showed that bile juice significantly slowed down the growth of cells, with 6000 and 2000 µg/ml showing the most significant inhibition. For SK-GT-4 cells, the inhibition reached 79.35% at 6000 µg/ml, while for AMGM cells, the inhibition was 67.54% at the same concentration. At lower concentrations (500 µg/ml), inhibition was minimal, with SK-GT-4 cells showing only a 7.85% inhibition and AMGM cells showing 2.02%. The IC50 values were calculated as 1674 µg/ml for SK-GT-4 and 1879 µg/ml for AMGM cells. The findings show that bile juice from the Iraqi Eastern Green Frog has strong anticancer properties, especially against esophageal cancer (SK-GT-4) and glioblastoma (AMGM) cells, with a clear response that depends on the dose.

Keywords: Iraqi frogs, bile juice, anticancer activity.

Introduction

Cancer remains a major global cause of illness and death, with both its medical and financial impacts on the rise. Esophageal cancer and glioblastoma, for example, contribute significantly to cancer-related

mortality (1). Cancer progression typically begins with mutations in genes that regulate cell growth, such as oncogenes or tumor suppressor genes (2, 3). This leads to unchecked and rapid cell proliferation, invading and destroying surrounding tissues and organs (4, 5).

Natural products, especially those derived from plants and animals, have played an essential role in drug discovery by offering a rich source of bioactive compounds that can target multiple biological pathways. This is particularly advantageous in alternative therapies, where the synergistic effects of these components may enhance therapeutic efficacy. Additionally, the traditional use of these natural products provides a preliminary foundation of efficacy and safety, which can guide further scientific exploration. There has been a notable rise in clinical trials focused on evaluating alternative and complementary therapies in recent years. These include non-drug interventions like exercise, meditation, and acupuncture, which are gaining recognition as valuable tools in holistic healthcare (6, 7).

Zootherapy, using animal-derived products in medicine, is particularly prominent in many traditional healing systems. However, studies and documentation on the use of animals in traditional medicine are very limited, particularly in Iraq. This practice draws upon extensive ethno-medicinal knowledge, where various parts of animals—such as skin, gallbladders, and urine—are employed to treat various ailments. For instance, a survey conducted in Nigeria identified 38 different animals used in treating diseases like diabetes, cancer, and asthma, highlighting the deep cultural and therapeutic significance of zootherapy in traditional medicines. You might find assistance in researching natural products and alternative medicine (8). In another study, animal-based healers harnessed the powers of nature to treat various refractory diseases. Six types of animals were used in treatments: fish, domestic animals, worms, leeches, larvae, and bees (9).

The present study aims to investigate the potential of Iraqi frog bile extract in inhibiting the activity of cancer cells. Two types of cancer cells, esophageal cancer and glioblastoma, were selected for this study.

Material And Methods

Collect the Bile juice of the frog

Bile juice was collected from the local Iraqi frog *Rana ridibunda* found in shallow pond waters according to the following steps: Frogs were gathered using clean plastic containers and brought to the laboratory. Notably, the frog found in Iraqi waters is known as the Eastern Green Frog, scientifically classified under the family Ranidae. They were thoroughly disinfected with iodine and alcohol. The frogs were

euthanized by severing the spinal cord at the neck, dissected, and their gallbladders were isolated. The bile juice was then extracted using a sterile syringe and placed in a Petri dish to dry in an incubator at 37°C. Once dried, the material was scraped off, collected into a 1.5 mL Eppendorf tube, and filtered using a 0.22µm syringe filter when preparing desired concentrations. The final product was stored at 4°C until further use.

Cell lines maintenance

The SKGT4 esophagus adenocarcinoma and AMGM glioblastoma cell lines were obtained from the cell bank of the tissue culture laboratory in the College of Education for Pure Sciences, Department of Biology, University of Basrah. The cell lines were maintained in fresh RPMI 1640 (US Biological, USA) culture medium, supplemented with 10% fetal bovine serum (Gibco, USA), 100 units/mL penicillin, and 100 µg/mL streptomycin, under humidified conditions at 37°C with 5% CO2. Once the culture reached the appropriate confluence (75% - 85%), cells were washed with Phosphate buffer solution (PBS) twice (Chemical Point, Germany), trypsinization by adding 1 ml of Trypsin-EDTA solution (Capricorn, USA), and resuspended in RPMI with 10% FBS in a new tissue culture flask, then incubated at the same above conditions. These sub-culture steps were returned every 2-3 days or when the culture density appeared under inverted microscopy as a monolayer (9).

Cytotoxicity assay

The cytotoxicity of the Bile juice of the frog was measured utilizing 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Bio World, USA). Cell viability was assessed using an amended colorimetric protocol according to the capacity of live cells to turn a tetrazolium compound into purple formazan crystals, by mitochondrial reductase enzymes (10). In brief, 1 $\times 10^4$ cells/well were seeded in a 96-cell culture plate and incubated for 24 hours at 37 ºC and 5 % CO2. SKGT4 cells and AMGM cells were subjected to different concentrations of Bile (500, 1000, 2000, and 6000) µg/ml for 72 hours. Then, 100 µl/ well MTT solution (10 µl MTT, 5 mg/mL of final concentration, and 90 µl serum-free media added to each well and incubated for 2 hours at humidified conditions. Formazan crystals were dissolved in DMSO and incubated for 15 minutes. The intensity of the color was measured using a Micro-tater ELISA plate reader at 620 nm. The number of viability cells was expressed as the percentage of control cells in extract medium-free serum (11)

The cytotoxicity percentage (%), was calculated as follows:

Cell viability = ($OD_{treatment}$ / $OD_{control}$) × 100 %, Where, $OD_{control}$ Optical density of untreated cells (control); $OD_{treatment}$ Optical density of treated cells (12).

Inhibition concentration (IC_{50}) determination

After three iterations of each experiment, identify each cancer cell line's inhibitory concentration (IC50). Data were collected from three technical iterations per concentration, and the inhibition rate equation was applied to absorption readings recorded by the ELISA Microtiter (Thermo Fisher Scientific, United States). GraphPad Prism Software (version 10.1.2 (324)) was used to obtain the IC50 values (GraphPad Software, San Diego, California, USA) (13).

This study demonstrated the cytotoxic properties of bile juice in suppressing the proliferation of SK-GT-4 and AMGM cancer cell lines. Findings from the MTT assay revealed that bile juice effectively inhibited the growth of SK-GT-4 and AMGM cells following 72 hours of treatment. Since bile juice demonstrated a dose-dependent effect, its inhibitory impact became more pronounced with increasing concentrations. The results of the current study, conducted using the MTT test, showed that the bile juice inhibited the growth of SK-GT-4 cancer cells at exposure to increased concentrations (500, 1000, 2000, and 6000) µg/ml for 72 hours. Each concentration was tested with three repeaters for treated and untreated cells (control group). The inhibition ratio at the highest concentration (6000 µg/ml) was 79.35%, while it was 78.95 % at the concentration (2000 µg/ml), while the lowest inhibition ratio (7.85%) was at the lowest concentration (500 µg/ml) with IC_{50} 1674µg/ml Figure 1. Similarly, glioblastoma cells AMGM showed a comparable level of inhibition to esophageal cancer cells SKGT 4, with the highest concentration (6000 µg/ml) resulting in a 67.54% inhibition. In comparison, it was 68.76% at the concentration (2000 µg/ml), while the lowest inhibition ratio (2.02%) was at the lowest concentration (500 µg/ml) with IC50 1879µg/ml Figure 2.

Discussion

Natural products exhibit remarkable diversity in their multi-faceted chemical structures, drawing significant attention to their role as modulators of biological functions. Consequently, they have been effectively utilized in drug discovery, contributing substantially to developing new therapeutics and leaving a profound impact on the field of chemical biology (14).

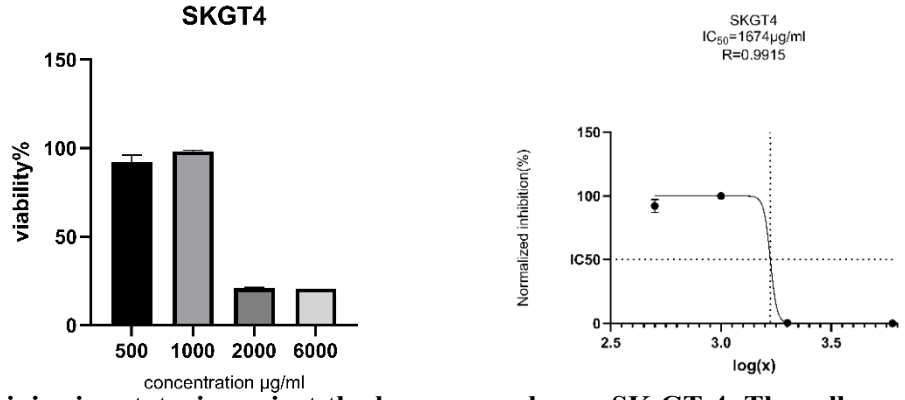

Figure 1. Bile juice is cytotoxic against the human esophagus SK-GT-4. The cells were treated with (500, 1000, 2000, and 6000 µg/ml) for 72 h. cytotoxicity was investigated using MTT. All data shown are mean ± SEM, p<0.05, N=4, from three independent experiments.

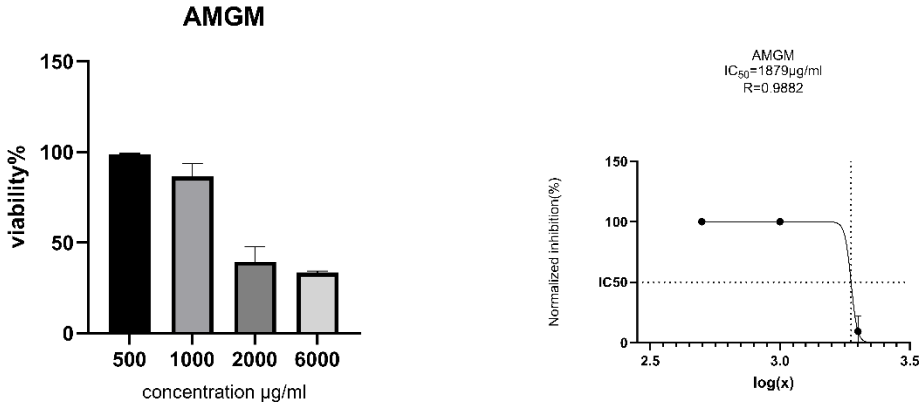

Figure 2. Bile juice is cytotoxic against glioblastoma cells AMGM. The cells were treated with (500, 1000, 2000, and 6000 µg/ml) for 72 h. cytotoxicity was investigated using MTT. All data shown are mean ± SEM, p<0.05, N=4, from three independent experiments.

Bile juice has emerged as a natural substance of significant interest in recent biomedical research. Studies have underscored its importance due to its diverse applications, particularly in treating neurological disorders. The bioactive components of bile juice have shown promising therapeutic potential, notably by attenuating programmed cell death and oxidative stress (15, 16).

The research showed that frog bile extract significantly slowed the growth of SK-GT-4 and AMGM cells after 72 hours of exposure to increasing concentrations or in a dose-dependent way. These concentrations were cytotoxic and may trigger cell death (17,18). Bile consists of various compounds, including bile salts, phospholipids, cholesterol, proteins, and bilirubin, all in stable equilibrium states (19). The primary constituents of Bile are bile acids, accounting for approximately 85% of the solid ingredients in Bile (20). These acids are important parts of cholesterol metabolites. They have been shown to slow disease progression and may have antitumor properties against different types of cancer cells, including tamoxifen-resistant breast cancer (21). The cytotoxic effect of bile juice may be due to inducing the trigger of apoptosis or potentially through mechanisms associated with membrane disruption (17, 18). Lithocholic acid (LCA) is one of the secondary bile acids that selectively induces apoptosis in both androgen-dependent and -independent prostate cancer cells without harming normal prostate epithelial cells (22, 23). This also applies to oral squamous cell carcinoma, cholangiocarcinoma, and hepatocellular carcinoma (21, 24).

The study suggested that frog bile extract can induce cell death in various cancer human cell lines; consequently, these novel Iraqi bile extracts show promising results as potent agents targeting different cancer cell types. Nonetheless, to better understand this agent, mechanistic insights into their activities remain to be substantially investigated. There is no precise report on the cost-effectiveness of preparing Bile and its derivatives. We believe that through further research into isolation, purification or synthesis, the expanse of bile Bile and derivatives can reduce, potentially making it more affordable for more cancer patients (25).

Conclusion

These findings highlight the potential of frog bile as a natural agent for cancer treatment. Future studies should focus on isolating and identifying the active compounds in the Bile, exploring their mechanisms of action, and evaluating their therapeutic potential *in vivo*.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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استكشاف الفعالية العلاجية لمستخلص الصفراء من الضفدع العراقي على نشاط خط الخلايا SKGT4وAMGM

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الخلاصة

لقد حظيت المستخلصات الطبيعية باهتمام كبير في علاج السرطان، خاصة في استهدافها تكاثر الخلايا السرطانية. بحثت هذه الدراسة التأثيرات السمية لعصير الصفراء المستخرج من الضفدع الأخضر الشرقي العراقي على خطي من الخلايا السرطانية وهي SK-GT_4 (سرطان المريء) وAMGM (سرطان الخلايا الدبقية). أظهرت هذه الدراسة وجود تأثيرات سمية للجرعة المعتمدة لعصير الصفراء من الضفدع الأخضر العراقي الشرقي على كلا من خطي السرطان SK-GT-4 وAMGM. أظهرت نتائج اختبار MTT ان عصير الصفراء يثبط بشكل كبير تكاثر الخلايا، حيث أظهرت اعلى التراكيز (6000مايكروغرام/مل و2000مايكروغرام/مل) تثبيطا كبيرا بالنسبة لخلايا SK-GT-4 وصل 79,35% عند الجرعة (6000مايكروغرام /مل) بينما لخلايا AMGM كان التثبيط 67,54% لنفس التركيز. وعند التراكيز المنخفضة (500 مايكرو غرام /مل) كان التثبيط ضئيلا حيث أظهرت خلايا SK-GT-4 نسبة تثبيط 7,85% فقط واظهرت خلايا AMGM تثبيطا بنسبة 2,02% فقط. تم حساب قيم IC50 على انها 1674 مايكرو غرام /مل بالنسبة الى لخلايا SK-GT-4 و1879مايكروغرام/مل لخلايا AMGM. تشير النتائج ان عصير الصفراء من الضفدع الأخضر الشرقي العراقي يمتلك نشاطا مضادا للسرطان بشكل كبير وخاصة ضد خلايا سرطان المريء SK-GT-4 وخلايا سرطان الخلايا الدبقية AMGM مع استجابة واضحة تعتمد على الجرعة.

الكلمات المفتاحية: الضفادع العراقية، عصير الصفراء، النشاط المضاد للسرطان.