Detection of DNA Damage in Esophageal Cancer Cell Lines Using Edible Insect Extract

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Abstract: This study investigates the genotoxic effects of an insect extract rich in bioactive compounds with potential anticancer properties. An extract of insects was made. In the biology labs at the University of Diyala's College of Education for Pure Science. GC-MS analysis was conducted at the Basra Oil Company laboratories to identify the active compounds in the desert locust (Schistocerca gregaria). The genotoxic effects were analyzed in esophageal cancer cell lines, and in comparison, to normal cell lines using the Alkaline Comet Assay method and a green dye. The experiments were done at the Al-Mustansiriya University Iraqi Center for Cancer Research. The findings showed that esophageal cancer cells had DNA damage, which stopped their ability to divide and caused apoptosis. However, normal cells are not harmed by the desert locust insect extract. With flavonoids, phenolic compounds, omega-3 alpha-linolenic acid, omega-6 linoleic acid, cholesterol, and glucosamine, the insect extract shows promise for the development of a successful treatment that uses natural insect extracts to eradicate esophageal cancer or any other type of cancer.

1 INTRODUCTION

Mutations caused by environmental factors in genes that regulate cell growth are what make cancer a deadly disease. Its main feature is what it does best: uncontrolled cell growth, which leads to the destruction of tissues around it[1]. Esophageal cancer has claimed the seventh ranking as one of the most common cancers, alongside being the sixth leading cause of cancer death globally [2]. This type of cancer is very advanced because it is hard for one to diagnose it early, and it spreads very quickly [3]. Esophageal cancer is broken down into two common types: squamous cell carcinoma and adenocarcinoma. The former is often the case of alcohol drinking and smoking, while the latter is attributed to gastroesophageal reflux disease [4]. Over the last decade, there has been interest in using insects as an alternative source of protein and other types of nutrients. In addition, they may be useful for the experimental treatment of cancer because of the many cytotoxic substances with anti-tumor activity that they produce. [5]. The large number of biologically active compounds produced by different insect species, which are important for their biological functions, has already aroused interest in pharmacology. A number of studies have shown various harmful effects on human cancer cells [6]. One study indicated that the viability of the Caco-2 colon cancer cell line can decrease after treatment with different concentrations of extracts from Schistocerca gregaria and Gryllus bimaculatus [5]. In the past few years, insects have become increasingly appealing for sourcing new bioactive compounds, particularly for therapeutic applications like cancer treatment. One of the most appealing is the desert locust (Schistocerca gregaria) due to its high nutritional value and rich pharmacological properties. Sterols, alkaloids, peptides, and numerous enzymes with potential protective functions against different infections, including colorectal cancer, are present in the bioactive profile of this traditionally used locust species [7].

2 MATERIALS AND METHODS

2.1 Extract Preparation

Schistocerca gregaria was collected and the species was authenticated by the Natural History Museum. To get rid of any contaminants, distilled water was used to wash the insects, and subsequently, the lower portions of the body were removed and disposed of. The insects were then dried and ground into a fine powder. Insects were extracted using a Soxhlet apparatus. 100 gm of the powdered insects was wrapped in a filter paper cone that was sharply closed to prevent any leakages. To the cone, together with the filter, 500 ml of the solvent was added for 24 hours. Thereafter, A rotating evaporator dial was used to remove a portion of the solution, leaving the necessary solvent left. [8].

2.2 Identification of Active Compounds

The biological tissues of Schistocerca gregarious, the gregarious phase locust, were transported to the Basra Oil Company laboratories for analysis. The active constituents were identified using the gas chromatography-mass spectrometry (GCMS) technique. The equipment used to analyze the insect's alcoholic extract included Agilent flame ionization gas chromatography (FID), gas chromatography-mass spectrometry (MSD) with a selective detector (GC-MS). The device's separation parameters along with the volume of extract injection are presented in Table 1 [9], [10].

2.3 Genotoxicity Assay

The genotoxic effects of DNA from the cell lines and agarose gels were studied using the Alkaline Comet Assay as described in [11]. Agarose gel was used to embed all malignant and healthy cells that had been exposed to varying doses of the insect extract of 1x105 cells/ml and diluted 1:10 (v/v) at 37°C. After the comet slices were prepared, the cells were lysed for an hour in a lysis solution at 4°C. Then, the samples were electrophoresed in a NaOH EDTA solution with a current of 21 volts for half an hour. After 30 minutes, the cells were counterstained with Syber green dye. As imaging was performed utilizing a Comet Assay software, the profiling data were analyzed through specialized software developed for Comet Assays.

2.4 Statistical Analysis

I used an unpaired t-test to analyze the data with a significance value of $P<0.05$ and presented the results as mean± standard error of the mean (SEM) in triplicate experiments calculated using GraphPad Prism 6 software as documented in [12].

Table 1 Settings for Separation Conditions of Desert Locust Extract gas chromatography coupled with mass spectroscopy (GC–MASS)..

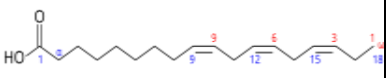
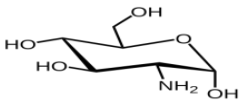
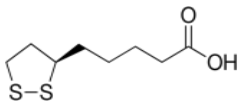
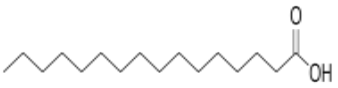
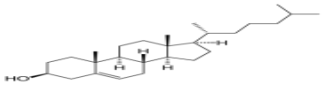
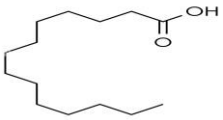
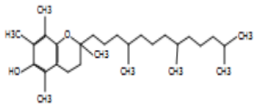
No	Device working	Device working information
1	Primary column temperature	C40
2	Final column temperature	C300
3	Rate of temperature rise	C/min 10
4	Ionization detector temperature	C 290
5	Helium carrier gas flow rate	Cm3/min 10
6	Total flow	ml/min 19
7	Column flow	ml/min 1
8	Cleansing flow	ml/min 3
9	Column type	HP-5MS 5 % Phenyl methyl siloxane
10	Column dimensions: length x inner diameter	M30 x m 0.25 x m 0.25
11	Injection volume	Lµ1
12	the pressure	Psi 7.0699

3 RESULTS AND DISCUSSION

3.1 GC-MS Analysis Results

By comparing the spectra with commercial spectral libraries, such as the Wiley GC/MS Library, the different parts of the insect extract were considered using the MassFinder Library and the Baser Library for essential oil components containing more than 3,200 authentic compounds with mass spectra and retention information. According to Table 2 and Figure 1, the results confirmed the presence of bioactive compounds in the insect extract, which is in line with findings from other studies [13].

Table 2: The active ingredients in the insect extract are displayed using the GC-Mass method.

No	Compound name	Chemical formula	Formula for structure
1	Hexadecanoic acid, methyl ester	C17H34O2	
2	9-Octadecenoic acid, methyl ester, (E)	C18H30O2	
3	Glucosamine	C6H13NO5	
4	Linolenic acid	C6H32O2	
5	;Hexadecanoic acid , methyleste	C16H32O2	
6	Cholesterol	C27H46O	
7	Tetradecanoic acid	C14H28O2	
8	Vitamin E	C29H50O2	
9	9,17-Octadecadienal, (Z)-	C18H32O	

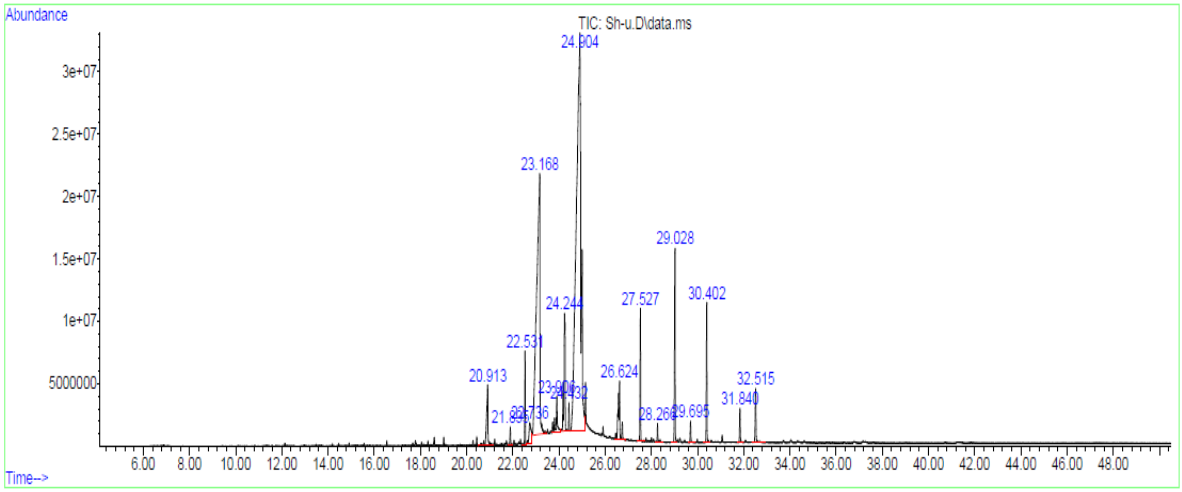


Figure 1: Gas chromatography-mass spectrometry of desert locust extract Schistocerca gregaria.

3.2 Genotoxic Effects of the Insect Extract on Cell Lines

Figure 2 demonstrates the genotoxic effects of the insect extract on esophageal cancer cell lines relative to normal cell lines., where: A) Represents esophageal cancer cells before treatment with the extract. B) Represents normal cell lines before treatment with the extract. C) represents esophageal cancer cells following administration of the extract at a 50 µg/ml dosage. D) depicts normal cell lines following treatment with the extract at a 50 µg/ml concentration. E) Represents an esophageal cancer cell in the apoptosis phase (programmed cell death). The Comet Assay demonstrated apoptosis in cancer cells treated with the insect extract, as indicated by the orange coloration of the cells, confirming their entry into the apoptotic phase. This apoptosis is likely due to the disruption of the mitochondrial electron transport chain, which is associated with reactive oxygen species (ROS) production within the cells [14]. Cell death and DNA damage may have been caused by caspase activity, especially caspase-3. The insect extract's free amino acids may be responsible for these effects.[15], [16]. No toxic effects were observed in normal cells treated with the extract. The extract's flavonoids and phenolic components probably caused cancer cells to undergo apoptosis by triggering antioxidants and preventing inflammation and migration. Consequently, these extracts may be utilized as adjuvants for anti-inflammatory, anti-oxidant, and anti-cancer medications [6]. The active compounds cause DNA damage or oxidation of nitrogenous bases, which leads to the death of cancer cells. In addition, these extracts work to prevent the division of cancer cells by inhibiting growth signaling pathways [17], [18]. Sterols like β-sitosterol, stigmasterol, and campesterol not only lower cholesterol levels, but also have possible effects in the modulation of cancer cells growth and differentiation [7]. Furthermore, the alkaloid trigonelline has displayed potent activity against tumor cells in colorectal cancer cell line by obstructing several important signaling pathways of cancer [19]. Schgr-AKH-II and other bioactive neuropeptides might control energy metabolism and cell proliferation, indicating further research could be warranted regarding cancer biology, Additionally, it has been demonstrated that a variety of proteins and enzymes derived from desert locusts include anti-inflammatory and antioxidant properties, which are crucial in regulating the growth of cancer. [20].

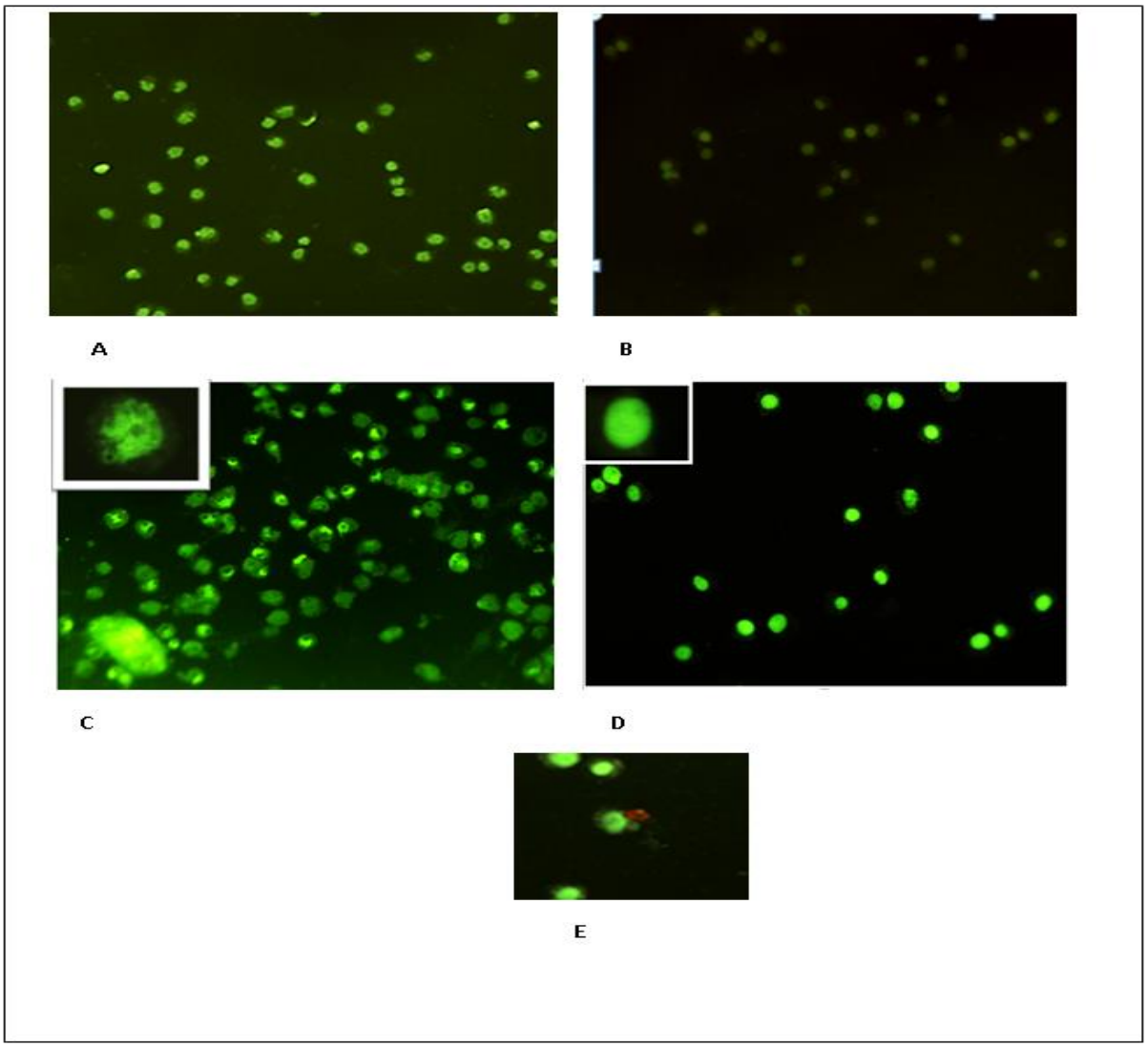


Figure 2: Illustrates the genotoxic effects of the insect extract on cell lines, where: A) Esophageal cancer cells before treatment with insect extract, B) Normal cells before treatment with insect extract, C) Esophageal cancer cells after treatment with insect extract, D) Normal cells before treatment with insect extract, E) A cancer cell in the apoptotic phase (programmed cell death).

4 CONCLUSIONS

The extract's treatment resulted in a promising reduction in esophageal cancer cell proliferation, suggesting the presence of bioactive compounds that may exhibit anticancer properties. This provides further evidence supporting the biological promise exhibited by the extract of the desert locust. The results also showed apoptosis induction in esophageal cancer cells, which further substantiates the hypothesis that the extract possesses properties of an antitumor agent. The extract also proved to show selective cytotoxic activity on malignant cells while leaving normal cells largely unscathed, reinforcing the notion that it could serve as a targeted therapeutic agent. More importantly, a decrease in genotoxicity markers was noted for the treated cells, suggesting that the extract may be free from direct genetic damage, which is an important factor in evaluating safety. Such evidence strengthens the rationale for isolating active extract constituents at the molecular level through further in vivo testing to assess efficacy, safety, and mechanisms of action. In conclusion, the evidence accumulated suggests further studies focusing on the desert locust are merited, especially considering the increasing demand for effective yet safe alternative cancer therapies. The presence of flavonoids, phenolic compounds, alpha-linolenic acid (omega-3), linoleic acid (omega-6), cholesterol, and glucosamine in the insect extract makes it a potential candidate for cancer treatment by inducing apoptosis, triggering antioxidants, and preventing inflammation and migration. These extracts might be added to anti-cancer medications as adjuvants , offering a natural alternative to chemical and radiation therapies that

also affect normal cells . These findings suggest that insect extracts may serve as promising adjuvants in anticancer therapies, offering a natural alternative to conventional treatments.

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