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In Vitro Antiproliferative and Wound Healing Effects of *Eurygaster maura* (Heteroptera: Scutelleridae) Metathoracic Scent Gland Secretion on HepG2 Cells

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Abstract: *The defense (scent) glands of species belonging to the order Heteroptera contain varying concentrations of aldehydes and hydrocarbons and squirt these chemicals during defense to protect themselves against the enemy. Odor gland analyses revealed different chemical structures among species and investigated whether they have an anti-cancer effect. It was aimed to reveal the effects of these chemicals found in the scent gland reservoir of Eurygaster maura species from the order Heteroptera on HepG2 liver cancer. The protective effect of E. maura odor gland secretions on HEPG2 cells was determined by forming control groups and treatment groups (1-25-50-100 µg/mL). The LD_{50} value was determined as 50 µg/mL. Increasing doses of odor chemicals prepared in DMSO solution were applied to dissociated HepG2 cells and antiproliferative effects were monitored at 12-24-48 hours intervals. The antiproliferative effect of Eurygaster maura on HepG2 cells was determined in control and treatment groups and the best activity dose was found to be 50 µL/mL. After incision, the first image of the formation of new cells in the medium was taken with a Zeiss inverted microscope. According to the results of the proliferation test, it was determined that the closure of the cells decreased depending on the amount of content obtained from the defense gland of Eurygaster maura.*

Keywords: *HepG2, Eurygaster maura, defense (olfactory) gland, anticancer.*

1. INTRODUCTION

Heteropterans, which belong to the Pentatomidae family, are also called stink bugs. The most important feature of these insects is that when they are stressed, they spray scented chemicals along with defense secretions from their scent glands [1]. The odor compounds they secrete are produced for defense purposes in adults as well as in nymphs, the young. The odor compounds they secrete are produced for defense purposes in adults as well as in nymphs, the young [2,3]. This mechanism developed by Heteroptera against their predators has been reported to be very effective in terms of defense [1,4].

Heteropterans feed by sucking plant sap. These species can live in a wide variety of areas. Heteropterans, which attract attention due to their large number of species and wide distribution among insect groups, also include many harmful species [5].*E. maura* is a pest species of the order Heteroptera that damages wheat plants and feeds on them. In insects of the order Heteroptera, defense glands have a more functional role. Stench glands are found in all species of Heteroptera, including adults and nymphs. The glands seen in nymphs usually open towards the back of the abdomen and are called "dorso-abdominal odor glands" (DAG). The odor glands observed in nymphs are located in both the abdomen and the thorax. The glands located in the thorax are called "metathoracic scent glands" (MTG) [6,7]. Adult individuals are located only on the thorax.

The abdominal glands in nymphs and adults are located towards the middle of the dorsal part [8]. The scent glands of Heteropterans open to the outside through three different openings on their abdomens.

These scent glands consist of 3 parts: anterior, median and posterior. Segment III of the abdomen has two slightly separated openings, while segments IV and V have a single opening [9]. Insects secrete chemicals from their scent glands as a defense, especially when startled and encountering predators [10]. Scent glands in heteropterans can vary in color and appearance. Odor glands are found in both males and females and are mostly different shades of yellow, orange and red [9].

The morphology of the scent glands varies depending on the insect's diet. In insects that are not in food shortage, a more swollen scent gland is observed, whereas in insects that are starving and in food shortage, it is small and malformed [10]. With the contraction of the muscles, the secretions spread over the fungus-like structures on the insect, so that the chemicals remain intensely in the environment. Insects behave according to the direction of the stimulus when excreting secretions. For example, they secrete unidirectionally if stimulated from one direction and bidirectionally if stimulated from behind [11,12].

According to the literature searches, the olfactory glands of *E. maura* have been analyzed in previous studies for both male and female individuals. The substance in which 15 different chemicals were present for both sexes of *E.maura* species was studied by gas chromatography-mass spectrometry (GC-MS). According to GC-MS in the literature, the highest amount of octadecanoic acid (31.15%) is found in *E. maura* female scent gland analysis, while the highest amount of n-tridecane (23.89%) is found in male scent gland analysis [14].

Liver cancer is one of the most common cancers and is the fifth most common cancer worldwide and the third leading cause of death [13]. Among hepatic cell lines, HepG2 cells were the first to exhibit the basic characteristics of hepatocytes. This lineage was isolated in 1975 and identified as hepatocellular carcinoma (or hepatoma, HCC). HepG2 cells were entered into the ATCC (American Type Culture Collection, Rockville, MD, USA) repository as a human cell line (HB 8065), obtained from liver tissue of a 15-year-old Caucasian male [15].

2. MATERIALS AND METHODS

2.1.Collection and Identification of Insects

The insects used in the study were collected on wheat plants at the edges of the field between June-September 2022 in Yozgat center. The insects were identified at the Department of General Biology, Faculty of Arts and Sciences, Department of Biology, YOBU.

2.2.Removing the Scent Gland From Insects

The collected insects belonging to *E.maura* species of the Pentatomidae family were dissected in PBS buffer under a binocular microscope. First, parts of the insects such as wings, legs, and head were removed and laterally divided into two parts with the help of forceps.Under a binocular microscope, the tissues around the olfactory gland in the thorax were removed and the olfactory gland was removed and given in different amounts to the implementation groups.

2.3.Preparation of Cell Culture

In this study, while investigating the antiproliferative effects of *E. maura* on liver cells, HepG2 cell line was selected as the cell line.HepG2 cell line RPMI was grown at 37°C in a humidified atmosphere containing 95% air and 5% CO_2.Permission for cell culture studies was obtained from Yozgat Bozok University, Faculty of Arts and Sciences, Biology Cell Culture Laboratory.

2.4. Antiproliferative Testing

HepG2 cells were suspended in RPMI medium at pH 7.4 and seeded in 6-well plates at a density of $5x10^5$ cells per well.After adhesion, the cells were expected to be 70% to 80% confluent, and then a single piece was dropped through a sterile 10 µL pipette tip and included in playts.To evaluate the anti-cancer effect of *E.maura* on HepG2 cells, control group and treatment groups (1, 25, 50 µL/mL) were formed.The effects on the proliferation rates around the opened scratch were monitored under an inverted microscope at 12-24-48 hours intervals and the initial proliferation distances and the scratch closure distances at 12-24-48 hours were imaged, and the dose with antiproliferative effect was determined by comparing these distances [15].

2.5. MTT Assays

MTT calorimetric analysis determines the properties of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which living cells are able to convert into an insoluble formazan precipitate.Tetrazolium salts can take electrons from oxidized substrates or from appropriate enzymes (e.g. NADH and NADPH). In particular, this is a consequence of MTT reduction in the ubiquinone, cytochrome b and c regions of the mitochondrial electron transport system and succinate dehydrogenase activity. This reaction results in blue formazan crystals in an organic solvent in which the yellow salts are soluble, forming a concentration that can be determined spectrophotometrically. Due to the many advantages of this test, it is considered a significant advance compared to existing conventional techniques.

3. RESULTS

When increasing doses of the defense secretions of *E. maura* were given to the implementationt groups and the control group on the proliferation of HepG2 cells, the proliferation rate of the cells decreased dose-dependently.When the implementationt groups of *E. maura* at increasing doses were compared with the control group, it was determined that the antiproliferative effect of 50 µg/mL dose was the best. The results are visually shown in Figure 1.

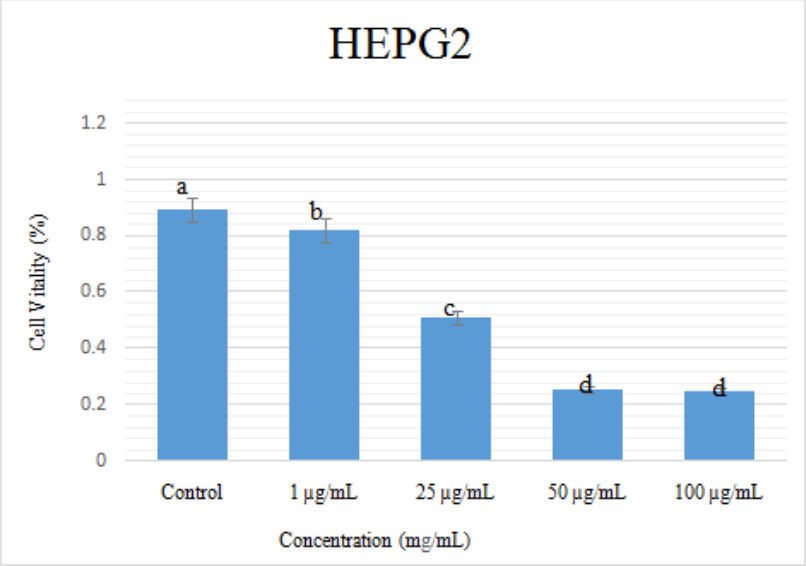

Figure1.*The effects of E. maura gland secretions on HEPG2 cell viability (Groups with different letters on the columns indicate significant differences (P<0.05) between. them).*

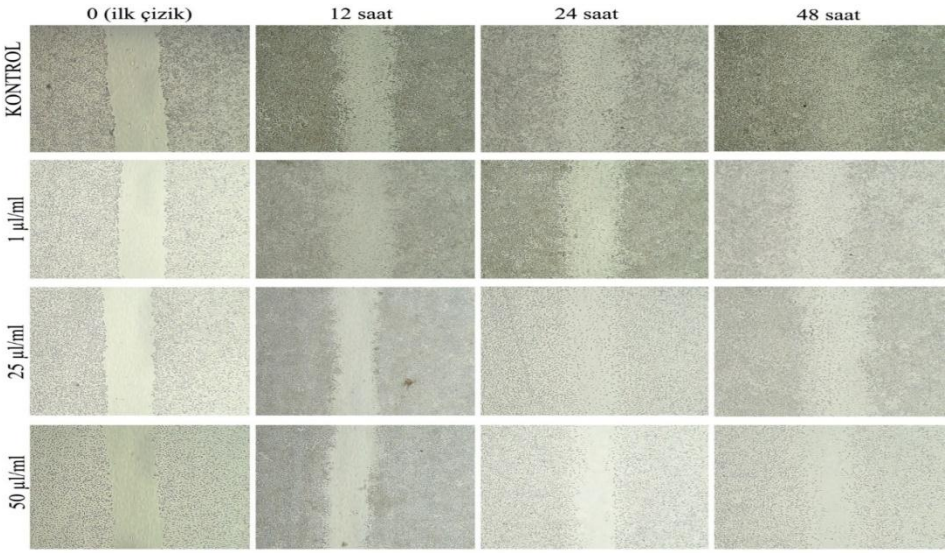

Figure2. *Inverted microscope images of HEPG2 cells.*

4. CONCLUSION AND DISCUSSION

The biochemicals secreted by the defense glands of insects are different and in very small quantities and contain various components.The components secreted by the defense gland have a primarily defensive role.In addition to these activities, this study may add anti-cancer properties to these secretions of *E. maura* species.Alternative ways are being sought by scientists against cancer, which is increasing day by day, and it should be evaluated in terms of public health that chemicals obtained from the defense glands of insects may also be effective.

Durak and Kalender (2009) and Chai et al. (2013) determined that the defense gland of *E. maura* species has 14 chemical substances as a result of gas chromatography-mass spectrometry analysis [16, 17]. The diversity of chemical constituents has been a source of light in these analyses by the researchers in our study. In addition, since there are not many difficulties in terms of field study, collection and identification of *E. maura* from the order Heteroptera, it is very important to investigate the anti-cancer effect of defense glands and to achieve success in terms of illuminating this issue. In addition, since there are not many difficulties in terms of field study, collection and identification of *E. maura* from the order Heteroptera, it is very important to investigate the anti-cancer effect of defense glands and to achieve success in terms of illuminating this issue.

In a study on *E. maura* scent glands, food for lizards was prepared from secretion extracted from the scent glands. Green lizards (*Lacerta viridis*) have been found to have repulsive responses to food, delay in approaching food, and a mixture of three aldehydes in the defense gland has a strong deterrent effect [18].

The biochemical components of the scent glands in such insects have many different properties on living organisms. The scent gland components of *Nezara viridula* (L.) have shown that (E)-2- decanal acts as a kairomone, thereby attracting female individuals to the eggs [19]. Heteroptera belonging to the order Pentatomidae:*Murgantia histrionica* and *Eurydema ventrale* were found to contain dialdehydes and diol esters, which act as attractive pheromones [20].

In this study, Heteroptera belonging to the order Pentatomidae: *Eurygaster maura* has a wide variety of scent gland chemicals and exists in a mixture. As a result of the literature review, fragrance gland components, whose anti-cancer properties have not been investigated, were applied on liver cancer and their antiproliferative effect was revealed.

Myrmicacin, found in the secretions of the leaf-cutting ant, was dubbed "the first insect-derived herbicide" because it could inhibit the growth of plant pollen germinating in ant colonies by blocking cellular mitosis.Myrmicacin was also found to inhibit mitotic progression at all stages, including metaphase and anaphase [21].

Hymenoptera: Formicidae, scientists investigated the chemistry of metapleural gland secretions and found the presence of bactericidal and fungicidal effects [22].

Hemiptera defense compounds have been identified as pheromone components for various species [23].

It has been found that the innate immune system present in *Drosophila* forms a senescence of antimicrobial peptides (AMP) with the fatty layer it provides in its body.Recently, studies showing that some cationic AMPs exhibit selective cytotoxicity against human cancer cells and enhance apoptosis on cancer cells have suggested that the innate immune system of *Drosophila* can suppress the progression of hematocyte tumors [24].

Researchers have identified Riptortus pedestris (Hemiptera: Alydidae), a pest of soybeans in Asia.Using this insect model, they observed that the secretion of *Riptortus salivary* glands has strong antibacterial activity against Burkholderia, which causes disease in humans and animals [25].

In this study, the anti-cancer properties of the biochemical components in the olfactory gland reservoir of *E. maura* were investigated by proliferation assay.As a result of the application of odor gland components to HepG2 cell line at increasing doses, it was determined that the best effect was shown at a dose of 50 µg/mL.When the control and implementent groups were compared in 12-24-48 hours microscope examinations, it was determined that the 50 µL/mL dose showed a significant inhibitory effect on cell proliferation.

As a result of the literature review of the current study, the effect of chemical substances found in insect defense glands on cancer cells is within the scope of an original study that has never been studied before, and it is thought that it will shed light on other studies to be carried out in the future and will form the basis for these studies.

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