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Investigating the Effect of Insect Body Extracts (Their Products) and Some Arthropods in Inhibiting the Growth of Cancer Cells or Tumours (Gastric cancer cell line/AGS)

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Abstract: Background and purpose: Arthropods including insects and Chilopoda and their products were used as medicine in ancient civilizations. Cancer is a burning disease that is one of the great problems of medical science, and no medicine has been discovered to control and suppress it. This research has investigated the effect of freeze-dried bodies and whole-body contents of some arthropods in inducing apoptosis and death of cancer cells (gastric cancer cell type AGS).
Investigation Method:6 species of insects: *Gryllotalpa* sp., *Polyrhachis* sp., *Dolichovespula* sp., *Apismellifera*, *Periplanetaamericana*, *Drosophilamelanogaster* and 1 species of Chilopoda *Scolopendra* sp. These were selected as samples.The samples were washed with distilled water and 70% alcohol, placed in a separate container, frozen and turned into powder. Then the powders were dissolved in 1% DMSO and in eight concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000, and 8000 µg/ml to control the AGS gastric cancer cell line in 6 replicates and 3 separate experiments. It was evaluated by the MTT test. IC50 and LD50 were calculated by Prism version 6 software to check the effect of concentrations determine the effective dose and choose the best extract to control the growth of AGS gastric cancer cells.
Results:(Pol) *Polyrhachis* sp. It had the best effect in controlling the AGS gastric cancer cell line.
Selected samples after *Polyrhachis* sp. They had a significant effect on the AGS cancer cell line, respectively: *Scolopendra* sp., *Dolichovespula* sp., *Apismellifera*, *Gryllotalpa* sp., *PeripelanetaAmericana*, and finally *Drosophilamelanogaster* had the least effect in inducing apoptosis and cell death in the cell line. Cancer cells had AGS, even at a concentration of 8000 micrograms/ml; the cell death did not reach 50% compared to the control. So, more tests can be done for the above samples.
Conclusion:The results of the application of 7 different species of arthropods and insects showed that these species have excellent therapeutic potential, especially in the treatment of AGS cancer cells.

Keywords: Gastric cancer, Entomotherapy, extract, cancer cells, AGS.

INTRODUCTION

Stomach cancer is the fourth most prevalent cancer globally and the second leading cause of cancer-related deaths (Farley et al., 2008). It is a complex and multifactorial disease that effects from a combination of infectious agents, environmental factors, and genetic predisposition (Zabalta et al., 2012).The northwest region of Iran has numerous incidences of stomach cancer, while the north, west, east, south, and central regions of Iran have varying levels of incidence. Ardabil, Zanjan, North Khorasan, West Azarbaijan, East Azarbaijan, and Razavi Khorasan are the six regions in Iran with the great incidence of stomach cancer. The mortality rate in this type of cancer is due to late diagnosis in the advanced phase of the disease (Lichtenstein et al., 2000).

Gastric cancer cells are formed on the inner lining of the stomach. As a result of metastasis, it spreads to the organs inside the abdomen, especially the liver, lungs, bone, abdominal wall and lymph nodes. In the first stages of the disease, there are no clinical symptoms, but after the passage of time, heartburn, pain and a feeling of heaviness in the upper abdomen, the sense of fullness in the stomach with pain before being fraught, nausea, loss of appetite, weight loss, the presence of blood in the stool and Black coloured stools, bloody vomiting when the tumour is located in the terminal region of the stomach, discovery of a palpable mass that indicates long-term growth and local spread of the tumour. Stomach cancer is the deadliest cancer in Iran. The cardia region of the stomach is the most common site of its occurrence, and it is difficult to treat, and most of the patients die after two years (Irvani, 2012).

Genetic and epigenetic changes are two important factors that can cause cancer. Inactivation of tumour suppressor genes and activation of oncogenes are the primary reasons behind this. Non-genetic factors that can lead to stomach cancer include the type of nutrition, alcohol consumption, smoking, hookah, severe hypertrophy in the folds of the stomach, Helicobacter pylori infection, stomach ulcers and some polyps, gastric atrophy, and surgery. Symptoms of stomach cancer can include difficulty swallowing, a sense of

bloating after eating, indigestion, unwanted weight loss, decreased stomach acidity, long-term consumption of smoked, dry and salty foods due to high nitrate, hot drinks, and genetics (Irvani, 2012).

When cancer appears, it often leads to the destruction of healthy cells, which can cause various toxic and side effects in patients. Therefore, finding new and effective alternatives to treat this disease is the need of the hour (Fock, 2014).
The expression of the hTERT gene, which is the catalytic subunit of human telomerase, is vital in tumor genesis and cancer progression. The mRNA level of this gene is directly linked to telomerase activity in cancer cells, which makes them immortal (Shay and Wright, 2010). This gene is not active in somatic cells, but is activated in embryonic and cancer cells (Ranji Najmeh, 2013).

Hypoxia, one of the key environmental features in solid tumors, is responsible for resistance to cancer treatment (Vaupel et al., 2007). Tumor cells lack oxygen due to a decrease in blood supply, yet they maintain their metastatic state and their ability to grow and reproduce (Ayremelo et al., 2015). However, they have an aggressive nature and resist conventional therapies such as radiation and chemotherapy (Hokel & Vaupel, 2001).
Stomach cancer often goes undiagnosed until it has advanced due to its lack of clinical symptoms. Surgery and chemotherapy are the most common treatments for gastric cancer, but they have a low efficiency rate, significant toxicity, and drug resistance (Lang et al., 2007; Wang et al., 2013; Amini Sarteshnizi et al., 2013).

Stomach cancer diagnosis is possible through endoscopy, biopsy or sampling, imaging such as CT scan and X-ray called barium swallow. Stomach cancer stage can be determined by blood test to check the function of vital body organs such as liver, endosonography, image tests. Imaging such as CT scan and PET scan, exploratory surgery to find signs of cancer metastasis is performed laparoscopically. The Stomach cancer is classified into 4 stages represented by Roman numerals I to IV. Unfortunately, Stage IV is the most advanced stage, indicating that the cancer has spread to other parts of the body. This can be a very challenging time for sick persons and their loved ones. Its treatment methods include: removal of tumors from the stomach wall in the early stages, taking away of a piece of the tummy or subtotal gastrectomy, complete gastrectomy or moving out of the entire stomach, removal of lymph nodes involved in the abdomen, surgery to relieve symptoms and signs, chemistry Radiation therapy with X-rays and protons, targeted drug therapy, immunotherapy or immunotherapy that these treatment methods should be According to Van-Custem et al. (2016), treatment methods such as surgery, radiotherapy, targeted drug therapy, and immunotherapy should be accompanied by supportive and palliative care. Accompanied by supportive and palliative care (Van-Custem et al., 2016).

In the past, various types of insects, such as silkworm larvae, grasshoppers, cockroaches, water bugs, etc., were used to treat certain diseases in different forms, such as food, injection, ointment, cooked and live insects (Costa-Neto et al., 2009; Carrera, 1993). The use of insects for treating diseases is called Entomotherapy (Blakney, 1999). In 1988, Namba and his colleagues listed 54 types of crude drugs obtained from insects in the herbal book. Chinese manuscripts mentioned 73 species of insects that were used to treat diseases (Chen, 1994|). Nowadays, 143 medicines are prepared and used from insects in China, which include 13 orders and 48 families (Zimian et al., 1997). In the 17th century, European people believed that many types of insects had healing power (Wigglesworth et al., 1976). For example, oil obtained from the larvae of *Melolonthavulgaris* L. is used to treat wounds and rheumatological conditions, as well as adult insects soaked in wine are used for anaemia treatment. For the treatment of earache and fainting, cockroach powder has been used (Ratclift et al., 1990). The use of insects in pharmaceuticals has come to the fore recently. Insects have the potential to be a game changer in medicine, says University of California professor Robert McClive. There are many effective antitoxins in the hemolymph of insects that are of interest to the pharmaceutical industry (Harpaz, 1973). In order to treat diseases such as malaria, dengue fever, sleeping sickness, and leishmaniasis, it is composed of protein, fat, carbohydrates, chitin, and melanin. Although entomotherapy is a traditional medicine, due to the use of insects, it is not widely known in today's world (Costa-Neto, 2009). Many researchers have taken note of their high capacity to reproduce and produce medicines and food in the past, with great importance being attached to them. By extracting and purifying glycoprotein lectin from the body of *Muscadomestica* larvae, researchers investigated its apoptosis-inducing properties in MCF-7 cancer cells and MLL-1 and MLL-2 from *Muscadomestica* bodies.

The anticancer effects of 200 microliters and 100 microliters have been demonstrated in several studies. In MCF-7 cancer cells, MLL2 is more effective at causing apoptosis and has no toxicity effects on normal human cells. An expensive study has shown that lectins found in plants have a therapeutic effect on cancer (Dossey, 2010). There are lectins from the fungi *Agaricusbisporus*, *Boletussativus*, *FlamulinaVelutipes* and *Ganodermalucidum* that have anticancer properties (Wang, 2020).

In vitro and in vivo studies have been conducted on *Drosophilamelanogaster* C-type lectin, which plays an important role in the body's immunity and antitumour properties (Wong, 2020). Bee venom, in the form of Apitox or HBV Ointment, has antimicrobial and bacterial properties which have been used for many years to treat arthritis pain and joint inflammation (Park et al., 2013). Against gram-negative bacteria, Bee venom is more effective than gram-positive type (Larivier&Melzack, 1996; Kown et al., 2002). Coriander honey prepared by bees has been represented to have antitumour and antioxidant effects on mouse EAC cancer cells in the study (Hegazi et al., 2014). The Brazilian bee, *polybiapaulista*, has an antimicrobial peptide that selectively inhibits the growth and spread of urinary, prostate, and leukaemia cancer cells. Peptide MP1 influences the phospholipids of cancer cell membranes, which change their permeability and kill these cells (Bueno et al., 2015). In chemotherapy, this mechanism is employed as an alternative treatment. It is essential to investigate the effects of different substances on the body of insects and

their products in the therapy of diseases, in particular cancer. The finding of new biologically active components that have unique action mechanisms and diversity in nature may provide us with chemical clues to the development of effective and affordable medicinal products (Elrayess& El-Hak, 2019).

Several insects have been identified in traditional medicine for their potential anti-cancer properties. The following insects have been studied for their potential to help treat certain types of cancer: *Apismellifera* and *Bombyxmori* for lung cancer, the Chinese horsefly with scientific name *Tabanusmandarinus* and the dada bug or *Cyclopeltaparva* for esophageal cancer, the red ladybug or *Hueckyssanguea* for skin cancer, *Blattaorientalis* or oriental cockroach for kidney cancer, and *Cryptotympana japonensis* or black cicada for thyroid cancer(Elrayess& El-Hak, 2019). The research aimed to evaluate the effectiveness of insect extracts, in controlling cancer cell growth, for example, the cancer cell line with epithelial origin is AGS. The research focused on the composition of the whole body of selected insects as the primary variable in the control of cancer cell growth.

MATERIALS AND METHODS

The current research was of an experimental type with a post-test design along with a control group, and in terms of the purpose, it was an applied basic research type that was conducted in a laboratory manner at the Islamic Azad University of Isfahan (Khorasgan branch) and the University of Isfahan.

In all stages, the research was carried out by the standard protocol for working with insects and cancer cells, as well as respecting ethical considerations. Seven types of arthropods and insects, which include 12 centipedes, 12 wasps, 25 bees, 60 flies, 12 American cockroaches, 25 ants or 25 white bees. The 25 numbers were chosen based on the previous research. The number of each insect varies according to its size and has been collected from different parts of Shahinshahr, Ziyar and Semirom province in Isfahan Province. The insects and arthropods collected have been cleaned, frozen dried and powdered after collection. After the extraction of the statistical population of cancer cells and MCF-7 in the number of 1x105 in a 96well plate in three repeated batches, then the number of 1x105 in a 96well plate in three repeated batches. MCF-7 cancer cells were purchased from Royan Research Institute. The scientific characteristics of MCF-7 cancer cells can be seen below.

Table 1: Characteristics of AGS cancer cells

AGS(C131)	Abbreviation and characteristics of cell line/cell extracted from tissue
Human/ stomach	animal tissue of origin cell extraction
Epithelial cells	, morphology and type of cell culture (adhesive, suspension)
DMEM + 10% FBS + Penestrep 1% + NEAA 1% + GLU 1%	culture medium used for cell proliferation and maintenance
Trypsin 0.05%	the proposed enzyme for cell passage and freezing
10%DMSO+90% FBS	type of freezing solution used for cell freezing

Preparation of insects and centipedes:

Mole cricket from the green space parks of Shahinshahr city, bees from beehives around Shahin-Shahr, dross flies from the wooden houses of Shahinshahr, centipedes from around Ziyar, American cockroaches from Shahinshahr parks, ants and yellow bee were collected from the slopes around the city of Semiram and placed in closed containers equipped with air inlets and outlets (for the survival of the insect) to be transferred to the freezer after identification. The samples were mechanically powdered and sieved (laboratory sieve 30 mesh). The final freeze-dried product is abbreviated as POL (*Polyrhachis* sp.), GRYL (*Gryllotalpa* sp.), API (*Apismellifera*), DROS (*Drosophilamelanogaster*), SCOL (*Scolopendra* sp.), PRIP (*Periplanetaamericana*), DOVES (*Dolichovespula* sp.) were named. In the final stage, insect powders were sterilized under ultraviolet light for 4 hours, and the powders a solution (RNA-free water and DMSO 1%) of the extract was prepared for injection into cancer cells.

Table 2: Names of species and families of insects that were used in the experiment

number	Insect name	order	family	scientific name	Abbreviation
1	Mole cricket	Orthoptera	Gryllotalpidae	*Gryllotalpa* sp.	GRYL
2	Ant	Hymenoptera	Formicidae	*Polyrhachis* sp.	POL
3	yellow wasp	Hymenoptera	Vespidae	*Dolichovespula* sp.	DOVES
4	Bee	Hymenoptera	Apidae	*Apis mellifera*	API
5	American cockroaches	Blattodea	Blattidae	*Periplaneta americana*	PRIP
6	Fruit Fly	Diptera	Drosophilidae	*Drosophila melanogaster*	DROS
7	Centipede	Scolopendromorpha	Scolopendridae	*Scolopendra* sp.	Scol

The process of preparing insect extract concentrations involved testing solutions of insect extract in different solvents such as distilled water, alcohol, ethanol and DMSO. After evaluating the turbidity and colour of the solutions, the best solvent was selected. It was found that 0.01 microliter DMSO was the most effective solvent for sterile powder

extracted from insects and centipedes. The medium was added to the powder, and concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000, and 8000 micrograms per millilitre were measured for injection into the well. Finally, AGS cancer cells were prepared.

MTT test or toxicity evaluation:

For the toxicity evaluation, a MTT test was conducted using 10 different treatments. The test was performed using 96-well plates containing cells, including no cells and empty medium without cells, which was pink in colour. The cancer cells used in the study grew 100% in this medium, without any additional substance. Eight treatments were tested, each with 8 concentrations of 62.5, 125, 250, 500, 1000, 2000, 4000, and 8000 µg/ml, and were repeated 6 times. The MTT test was done three times per cell, over three weeks, and the absorbance was measured by a micro plate reader (Bio-Rad) at 570 nm. The untreated AGS cells were considered as a control with 100% viability, and the culture medium was pure. The results were expressed as mean and standard deviation values from four independent measurements. The cell viability population (percentage) was obtained using the following equation, and compared to the untreated control group.

100× (OD570 (control)) / (OD570 (sample)) = cell viability (%)

Calculation of IC50:

Statistical analysis was conducted using Graph Pad Prism V.6 software to determine the half-maximal inhibitory concentration (IC50). The IC50 was obtained through logarithmic non-linear regressions (inhibitory) versus response with four parameters. The upper and lower parameters were limited to 100% and 0%, respectively, for the calculation of IC50. To estimate the 50% lethal dose (LD50) in the human body in the laboratory, the IC50 values were used in a formula:

[log LD50 = 0.372 × log IC50 (µg/ml) + 2.024]

Findings:

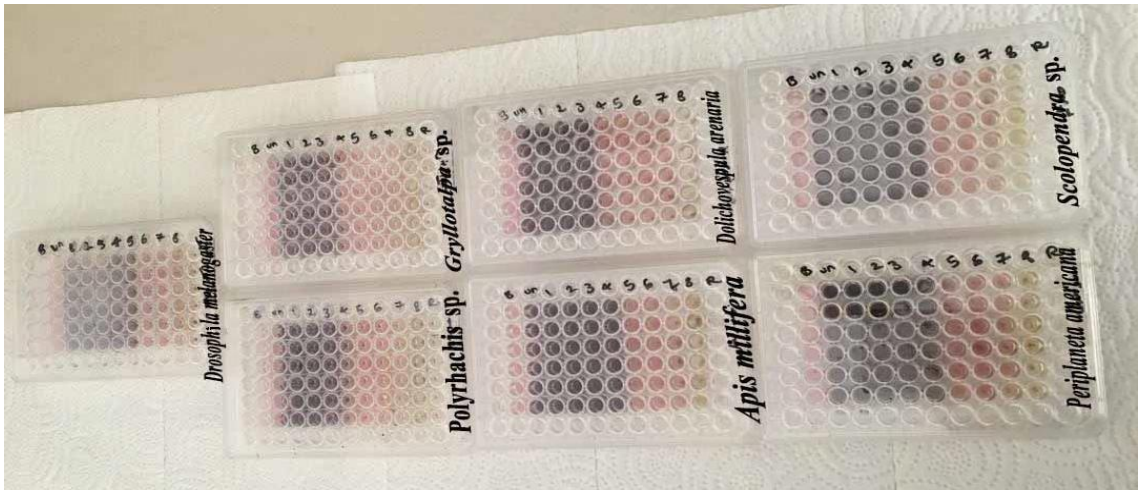

Figure 1: Evaluation of the toxicity of extracts of *Gryllotalpa* sp., *Polyrhachis* sp., *Dolichovespula* sp., *Apismellifera*,*Periplanetaamericana*, *Drosophilamelanogaster* and *Scolopendra* sp. on the AGS cancer line

***Apismellifera*:** After conducting the MTT test on the *Apismellifera* sample, it was observed that a concentration of 62.5 µg/ml slightly increased the growth of cells, which indicates that an inappropriately low concentration of the drug can have the opposite effect in improving the disease. On the other hand, doses of 125, 250, and 500 micrograms/ml resulted in a gradual decrease in the growth of AGS cells. Furthermore, a significant decrease in the growth of AGS cancer cells was observed in concentrations ranging from 1000 to 8000 micrograms/ml. Among these concentrations, 8000 micrograms/ml showed the best and greatest effect on controlling the growth of AGS cells.

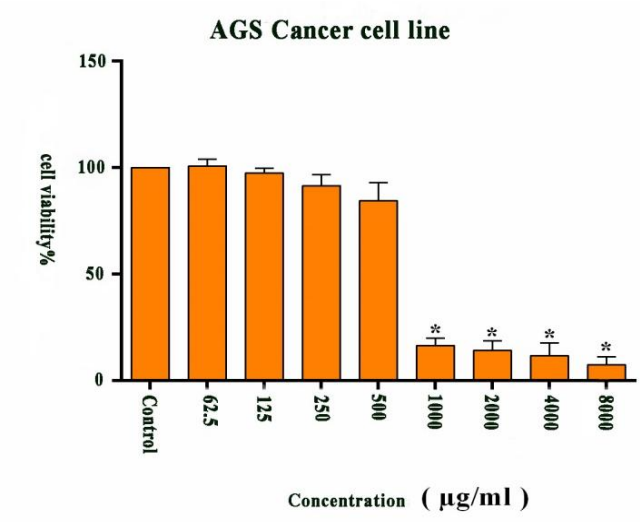

Diagram 1: MTT test, *Apismellifera* on AGS cell line

Peripelaneta americana:

At a concentration of 62.5 µg/ml, the *Periplanetaamericana* extract sample slightly enhances the growth of AGS cells. However, in the subsequent two series, the growth of AGS

cells is gradually reduced and controlled, starting from a concentration of 62.5 up to 1000 micrograms/ml and ultimately, from 2000 to 8000 micrograms/litre. A significant control of the growth of AGS cells is observed. The highest potential for inducing apoptosis in AGS cells is seen at a concentration of 8000 micrograms per millilitre.

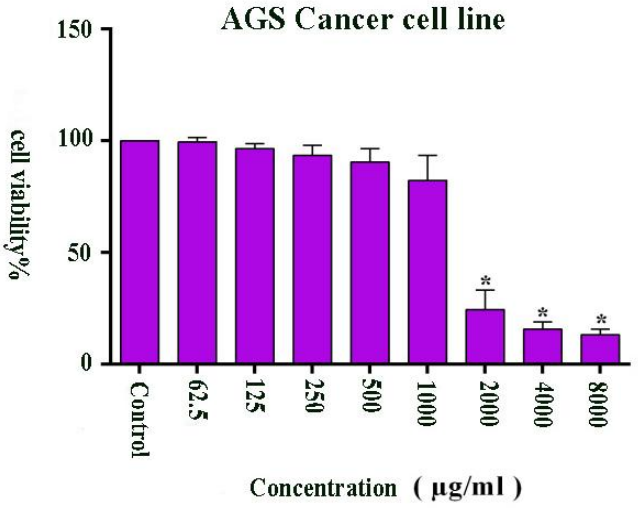

Figure 2: MTT test, *Periplanetaamericana* on AGS cell line

***Dolichovespula* sp.:**

The results show that at a concentration of 62.5 µg/ml, the growth of AGS cells is decreased. When compared to this concentration, concentrations of 125 and 250 micrograms/ml demonstrate a significant reduction in AGS cancer cell growth and from concentrations of 500 to 8000 micrograms/ml, there is an increase in the intensity of apoptosis and cell death induction. Notably, the concentrations of 4000 and 8000 micrograms/ml have the most significant control over the growth of AGS cancer cell lines, respectively.

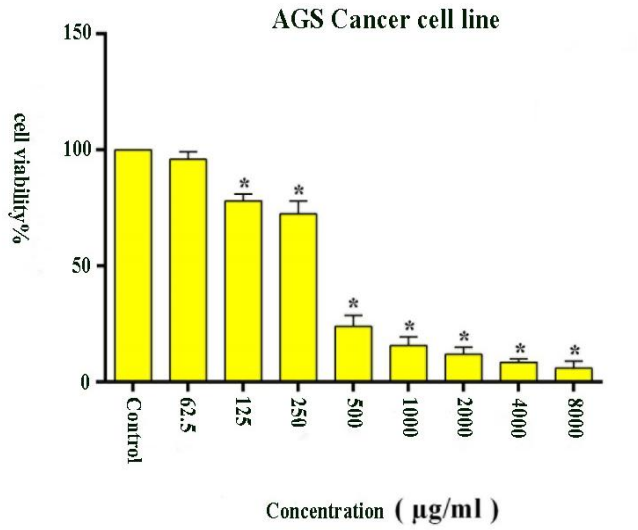

Figure 3: MTT test, *Dolichovespula* sp. on the AGS cell line

***Gryllotalpa* sp.:**

In the *Gryllotalpa* sp., the growth control of AGS cells becomes more or less visible at a concentration of 62.5 µg/ml.

There is a significant decrease in a concentration of 125 µg/ml in growth compared to 62.5 µg/ml. This growth control is also more visible in AGS cells at a concentration of 250 µg/ml compared to 125 µg/ml. The significant induction of apoptosis occurs at concentrations of 500 and 1000 µg/ml in the AGS cancer cell line, which is significant, compared to the concentration of 250 µg/ml. The induction of apoptosis and cell death is almost the same at concentrations of 500 and 1000 µg/ml and is very significant. From the concentration of 2000 to 8000 micrograms per millilitre, there is a gradual decrease in the viability of AGS cells, which is significant compared to the concentration of 1000 micrograms per millilitre. The greatest effect on the induction of apoptosis and cell death occurs at a concentration of 8000 µg/ml.

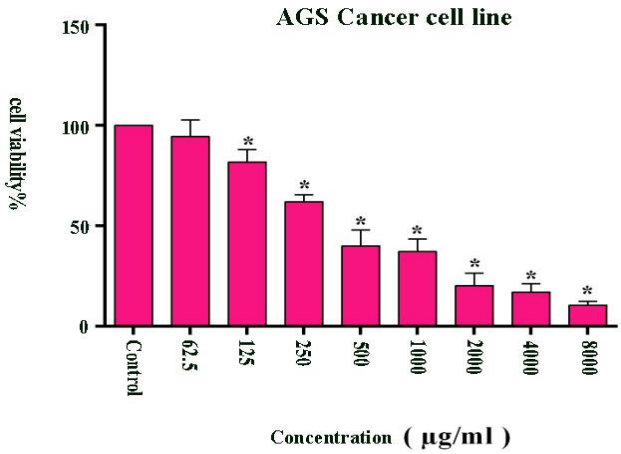

Diagram 4: MTT test, *Gryllotalpa* sp. on the AGS cell line

***Polyrhachis sp.*:**

After conducting an MTT test and normalizing the results, it was discovered that a sample of *Polyrhachis* sp. is highly effective in controlling the growth of AGS cancer cell lines and inducing apoptosis. The viability of the AGS cancer cell line decreased significantly at a concentration of 62.5 µg/ml, with the greatest effect observed at a concentration of 8000 µg/ml. This reduction of approximately 50% viability at a concentration of 62.5 µg/ml compared to the control, which has a viability of 100% in AGS cells, is significant.

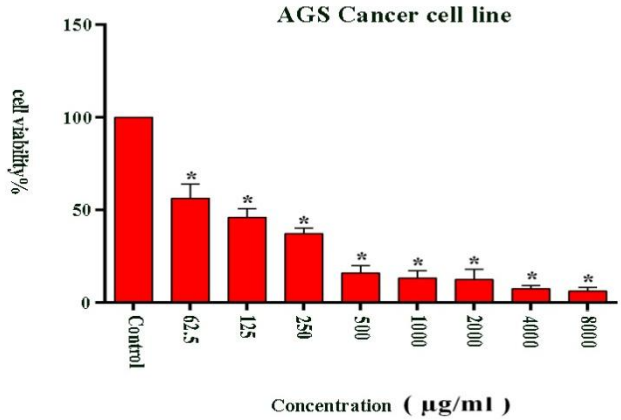

Diagram 5: MTT test, *Polyrhachis* sp. on the AGS cell line

Drosophila melanogaster:

Results show that concentrations ranging from 62.5 to 500 µg/ml did not significantly reduce the viability of the cancer cells compared to the control group. However,

concentrations ranging from 1000 to 8000 micrograms/ml resulted in a significant decrease in the viability of the cancer cells compared to the control group. It is worth noting that even at a concentration of 8000 micrograms/ml, the percentage of survival of AGS cells remained above 50%, which is not significant.

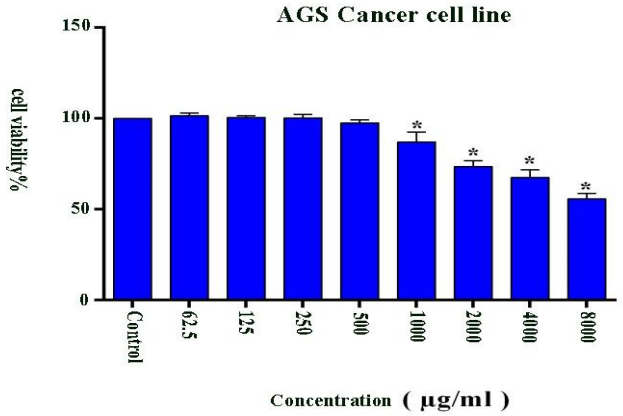

Figure 6: MTT test, *Drosophilamelanogaster* on AGS cell line

Scolopendra sp.:

After conducting analysis and normalizing the survival percentage of AGS cancer cells in the Scolopendra sp. sample, the study found that there was no significant reduction in the survival percentage of cancer cells at concentrations ranging from 125 to 500 micrograms per millilitre when compared to the control group. However, when concentrations were increased to 1000 to 8000 micrograms per millilitre, a significant decrease in the survival percentage of cancer cells was observed. It is worth noting that from the concentration of 1000 to 8000 µg/ml, the survival percentage of AGS cancer cells is more or less the same and below 10%. In simpler terms, the study showed that the Scolopendra sp. sample at concentrations ranging from 125 to 500 micrograms per millilitre did not significantly affect the survival of AGS cancer cells. However, when concentrations were increased to 1000 to 8000 micrograms per millilitre, there was a noticeable decrease in survival percentage, with all concentrations in that range being below 10%.

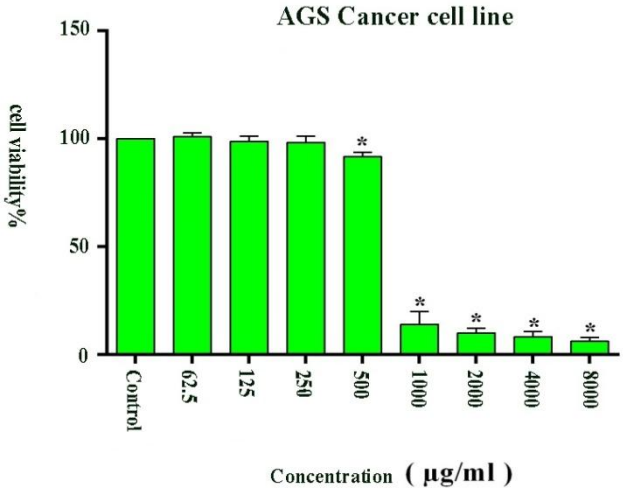

Diagram 7: MTT test, *Scolopendra* sp. on the AGS cell line

Comparison OfMTT Test Of All Insect Extracts On AGS Cell Line:

Scientific research was conducted to compare the effects of insect extracts on the AGS cell line using the MTT test. The report suggests that the *Polyrhachis* sp. sample had a significant impact on reducing the growth of the AGS cancer cell line, even at a concentration of 62.5 µg/ml. The sample also induced apoptosis in the AGS cancer cell line by nearly 50% at the lowest concentration, which was visible up to a concentration of 1000 micrograms/ml when compared to other samples. From the concentrations of 2000 to 8000 micrograms/ml, the effect of the *Polyrhachis* sp. sample was similar to the samples of *Scolopendra* sp., *Apismellifera*, and *Dolichovespula* sp.

Moreover, the report highlights the results of the MTT test and cell viability, indicating that the *Polyrhachis* sp. sample had the most significant impact and a substantial decrease in the viability of the AGS cancer cell line, while the *Drosophilamelanogaster* sample had the least effect.

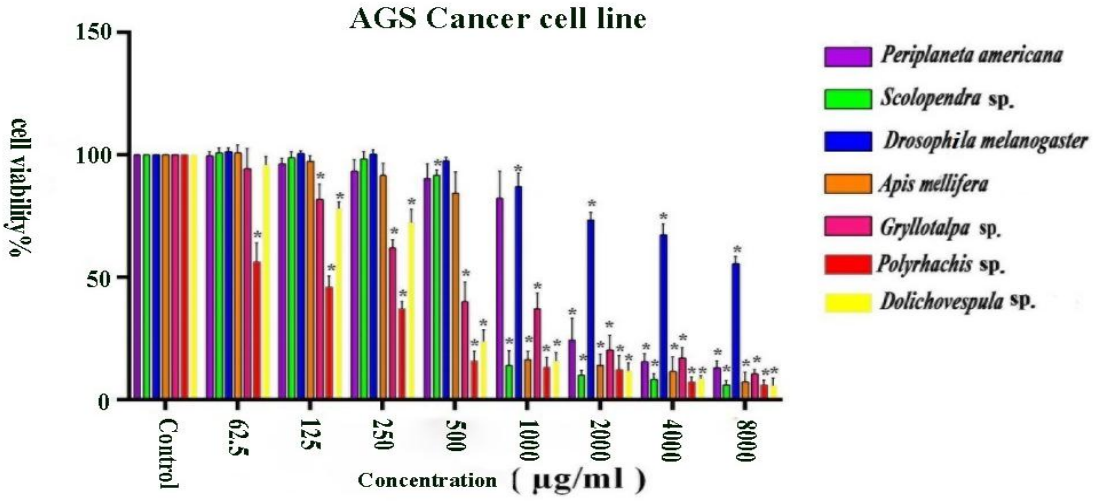

Diagram 8: MTT test comparison of all insect extracts on AGS cell line

DISCUSSION AND CONCLUSION

This research has made significant progress in the field of cancer treatment. The study found that medicine based on the biological basis of insects and arthropods has great therapeutic potential, which is more affordable for patients due to the abundance of these organisms. The researchers tested 7 different genera of arthropods and insects for their therapeutic potential on AGS cancer cells and found that *Polyrhachis* sp. had the best effect on the cell line, even at low concentrations. The other samples that had a significant effect on the AGS cancer cell line were *Scolopendra* sp., *Dolichovespula* sp., *Apismellifera*, *Gryllotalpa* sp., *Peripelanetaamericana*, and finally *Drosophilamelanogaster* least effect in inducing apoptosis and cell death in the cell line. However, even at a concentration of 8000 µg/ml, the cell death did not reach 50% compared to the control, so more tests can be done for the above samples. It was found that *Scolopendra* sp. was significantly effective in inducing apoptosis and cell death in AGS cancer cell lines even at low concentrations, with a concentration of 125 µg/litre having a good effect. The study suggests that arthropods, especially centipedes and insects, have good therapeutic potential in improving human diseases, especially cancer. Further investigation and testing should be done to explore the therapeutic properties of these samples.

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