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Investigation Add Effect Hexane Extract of *Juncus Rigidus* Roots on Human Dermal Fibroblast Cell Line (DHSF)

Ali Mohammed Hussein[1*], Alaa Ramthan Hussein [2] and Hatem Karim Jassab [3]

1 Babel Education Directorate, Kutha Education Department, ali.mh.1987.com@gmail.com, 51009, Babylon, Iraq.
2 Thi-Qar Education Directorate, Al-Rifai Education Department, alaathiqar57@gmail.com, 57009, Thi-Qar, Iraq.
3 Babel Education Directorate, Kutha Education Department, hhkk65130@gmail.com, 51009, Babylon, Iraq.
***Corresponding author email:** ali12.ooa12@gmail.com; mobile: 009647740826958

دراسة تأثير اضافة مستخلص الهكسان لجذور نبات الاسل *juncus rigidus* على خط الخلايا الليفية الجلدية البشرية DHSF

علي محمد حسين كاظم[1*]، علاء رمثان حسين[2]، حاتم كريم جساب[3]

1 مديرية تربية بابل، قسم تربية كوثا، ali.mh.1987.com@gmail.com، 51009، بابل، العراق
2 تربية ذي قار، قسم تربية الرفاعي، alaathiqar57@gmail.com، 57009، ذي قار، العراق
3 تربية بابل، قسم تربية كوثا، hhkk65130@gmail.com، 51009، بابل، العراق

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ABSTRACT

Background:
The study examined the effect of the hexane extract from *Juncus rigidus* roots on the human dermal fibroblast cell line (DHSF) with the aim of exploring and identifying safe therapeutic alternatives for normal cells.

Materials and Methods:
The active compounds (phenols and flavonoids) in the plant extract were quantified, yielding (15.87, 17.33) mg/g, respectively. The DPPH assay, using concentrations of (20, 30, 40) mg/ml, recorded inhibition rates of (21.3, 23.44, 26.24) %, respectively. The amount of reactive oxygen species (ROS) in the root extract was found to be very low, measuring 343 nml. The extract was tested on normal cells of the human DHSF line at five concentrations (25, 50, 100, 200,400) µg/ml and for three durations (24, 48, 72) hours.

Results:
The highest cytotoxicity rate was recorded at a concentration of 400 µg/ml, reaching 34.69% after 72 hours of exposure to the extract, with significant differences observed between the higher concentrations at a probability error rate of $P \leq 0.05$. In contrast, lower concentrations showed less inhibition of the cells, with the lowest concentration of 25 µg/ml resulting in a minimal rate of 3.03%. No significant differences were observed between the lower concentrations at exposure times of 24 and 48 hours.

Conclusion:
The extract is relatively safe for normal DHSF cell lines even at the highest concentration used in the experiment, with the highest cell mortality rate being after three days of exposure.

Key words: *Juncus rigidus* ; DHSF ; Hexane extract ; DPPH ; Cytotoxicity

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INTRODUCTION

The knowledge about medicinal plants and their preventive and therapeutic importance has accumulated over generations, with this information being passed down along with newly discovered experiences regarding compounds that have a direct impact on human health [1]. Juncus rigidus (JR) is a widely spread medicinal plant known for its resilience to harsh environmental conditions such as salinity and drought. It belongs to the Juncaceae family, which is characterized by high tolerance to water and salt stress. From these extreme environments, JR has developed unique secondary metabolites with therapeutic properties. Among the compounds that JR is known for are phenanthrenes, which have distinctive properties. In addition to natural antioxidants, they play a role in eliminating reactive oxygen species (ROS) [2].

Despite the widespread use of synthetic chemical compounds in treatment and their proven efficacy on various health aspects for patients, the emergence of side effects from these treatments has posed significant challenges. For example, cortisone medications used in the treatment of skin diseases can cause skin thinning, increasing susceptibility to sun exposure and making the skin more prone to bruising and cracking. Currently, there are many topical treatments, such as ointments and moisturizers, that contain cortisone in their composition. With repeated use on specific areas of the skin, numerous problems can arise, such as microbial infections due to immune suppression, inflammation of hair follicles, and changes in skin color [3].

Many medicinal plants have been tested to find alternative treatments with fewer side effects. Among these plants is JR, which has been studied for its various health benefits due to its compounds with numerous pharmacological effects. It has proven effective in eliminating free radicals, acting as anti-tumor agents, and treating inflammatory infections on the skin without any side effects compared to synthetic drugs [4].

Some studies have indicated the potential use of extracts from parts of the Juncus rigidus plant, such as roots, leaves, and stems, as alternatives to synthetic formulations containing cortisol for the treatment of skin diseases like eczema and psoriasis. This is due to the plant's promising compounds in this field, which exhibit minimal or no cytotoxicity to skin cells compared to chemical formulations. This has led to the search for safe compounds for the same purpose [5].

MATERIALS AND METHODS

- **Plant collection, extraction and estimation of active compounds**

The plant samples were collected from local areas in the northern part of Babil Governorate in June. They were thoroughly washed with water to remove any adhering soil, and the roots were separated from the plant after thorough washing and left to dry in the shade. Once dried, the roots were ground using an electric grinder until they became a fine powder. A quantity of 50 grams of the plant powder was used and placed in hexane solvent to prepare the extract according to method [6]. Additionally, the DPPH test was used to determine the antioxidant activities of the root extract as described in [7], along with an estimation of the total ROS content in the extract.

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- **Testing of root extract in normal DHSF cell line**

After preparing the hexane extract for laboratory use according to [8], the following concentrations were prepared (25, 50, 100, 200, 400 µg/ml) . The DHSF normal cell line, derived from human skin, was prepared in the laboratory following the steps outlined in [9]. The extract was then tested on the human cells using the pre-prepared concentrations and exposure periods of 24, 48, and 72 hours. The MTT assay was used according to the previous source. Results were read using an ELISA reader at a wavelength of 490 nm to determine the impact of the JR root extract on the normal cell line.

RESULTS AND DISCUSSION

After conducting analyses on the plant extract using hexane as an organic solvent for the roots of JR, significant amounts of bioactive compounds were found. Flavonoids and phenols were recorded at 17.33 mg/g and 15.87 mg/g, respectively. The DPPH antioxidant activity test showed inhibition rates at concentrations of 20, 30, and 40 mg/ml with 21.3%, 23.4%, and 26.2% inhibition, respectively, while the total ROS content test yielded a very low result of 343 nml. As noted in [10], attention has shifted toward alternative medicine research and medicinal plants, among which JR stands out for its unique compounds, considered a taxonomic characteristic of the plant. The most notable of these compounds are phenanthrenes and other naturally occurring active compounds formed as secondary metabolites. These compounds are produced in response to the plant's harsh environment, including water and salt stress, and exhibit strong activity against oxidative agents in vivo, especially at sites of inflammation and infected wounds

There are numerous studies on therapeutic alternatives in medicinal plants. For example, [11] states that *Juncus* possesses significant compounds that support the immune system's ability to combat fungi and skin bacteria and accelerate wound healing. It also reduces the incidence of localized gum inflammation. The plant extract has a protective role in minimizing inflammatory irritation of the epithelial lining of the mouth caused by invasive microbes. The study further confirms that JR products are safe for normal cells, suggesting they could serve as a natural alternative to manufactured treatments for maintaining oral and skin health.

Some chemical compounds with their biologically active derivatives such as phenanthrenes and hydroxyphenanthrene have been isolated from plant extracts. João et al [12] indicated the protective role of alcoholic and aqueous extracts of the plant against opportunistic bacteria, as they have proven effective in treating and repairing damaged tissues due to inflammatory suppurations resulting from opportunistic bacterial infections such as Staphylococcus aureus and Escherichia coli associated with wound and burn inflammation. There is also a major role for JR extract as an anti-proliferation of some skin fungi such as Candida and Aspergius fungi.

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The results of the cytotoxicity test, as shown in Table (1), indicate that the DHSF cells used in the experiment were not affected by the extract at a concentration of 25 µg/ml for all exposure periods (24, 48, 72 hours), with no significant differences observed between the exposure times, as seen in Figure (2). However, a slight effect began to appear at a concentration of 50 µg/ml, where the cell inhibition rate was very low after 48 hours of treatment, while the percentage of cell death was 10.03% for the same period, with no significant differences noted between the exposure times of 48 and 72 hours.

At higher concentrations of 100 and 200 µg/ml, significant differences began to emerge, with the percentage inhibition of DHSF cells reaching 22.23% and 29.82%, respectively, after 24 hours at a significance level of 0.05. The highest percentage of inhibition of normal cells in the DHSF cell line was recorded at the largest concentration of 400 µg/ml, reaching 34.69% after three days of treatment with the extract, as shown in Figure (3).

Table (1) Effect of hexane extract of JR roots on DHSF cell line for exposure times (24, 48, 72) hours.

Con / time	25	50	100	200	400
24	0.5 ± 3.03	0.6 ± 5.41	0.91 ± 13.84	1.03 ± 22.43	1.32 ± 29.23
48	1.66 ± 5.44	1.12 ± 10.0.3	0.89 ± 16.92	1.23 ± 26.06	1.24 ± 32.26
72	1.26 ± 5.89	2.11 ± 12.21	1.99 ± 19.72	3.12 ± 29.03	2.03 ± 34.69
LDS 0.05	2.038				

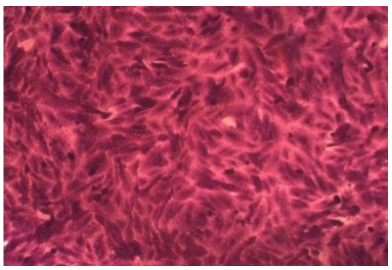

Figure (1) Normal cells not exposed to the extract

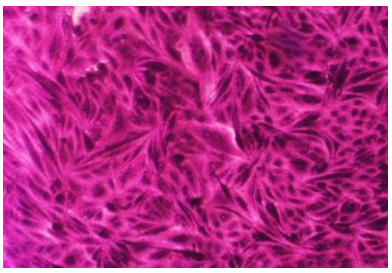

Figure (2) Cells exposed to the extract after 24 hours of exposure

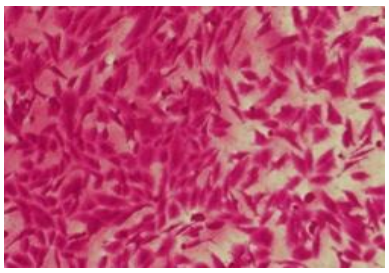

Figure (3) Cells exposed to the extract at a concentration of µg/ml (400) and an exposure period of 72 hours.

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The results are consistent with [13] in indicating that the plant is safe for normal cells while having toxic effects on cancerous cells. It was tested on some cancer cell lines, including cervical cancer Hela cells and human T-47D cell line, which showed high inhibition rates against cancer cells. The study also confirmed that the plant extract is of low toxicity to the normal MRC-5 cell line, supporting the results obtained.

The results of study confirmed that plant has a great antioxidant activity and that it does not contain harmful reactive oxygen species (ROS) except for very small amounts, which was confirmed by plant chemistry tests and that it is of little toxicity to normal cells. The study of [14] indicated the amount of active compounds in the extracts of the roots of the Juncus rigidus plant and their role in antioxidant activities. This supports what was stated in the results of the current study, as the hexane extract contains amounts of flavonoids and phenols. The same study also confirmed what was obtained from the antioxidant activity test, as the plant has the effectiveness of scavenging free radicals that accumulate in some areas of skin tumors and damaged tissues in wounds, leading to increased local inflammation.

Some chemical compounds and their active derivatives, phenanthrene and alpha-hydroxyphenanthrene, were isolated from the extract of the Juncus rigidus plant and were found to be effective against cancerous tumors by activating the body's immune system to recognize abnormal cells and thus distinguish and eliminate them at the same time. Research has shown that these compounds, despite their high activity against tumor cells, are of low toxicity to normal cells [15].

CONCLUSIONS

The roots of JR contain compounds with interesting activities that effectively remove free radicals in living tissues. There is no significant cytotoxicity to normal cells when using the extract at the concentrations applied in the study. It is also relatively safe for DHSF cells, and there is an inverse relationship between the viability of using the extract and the concentration used. JR is considered a plant utilized in pharmaceutical and alternative medicine research to develop safe drugs for living tissues.

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Conflict of interests:

There are non-conflicts of interest.

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الخلاصة

المقدمة:

تناول البحث اختبار تأثير مستخلص الهكسان Hexane لجذور نبات الاسل Juncus rigidus على خط خلايا الليفية الجلدية البشرية المشتقة من أدمة الجلد (DHSF)، وذلك بهدف البحث والتحري عن بدائل علاجية آمنه على الخلايا الطبيعية.

طرق العمل:

تم تقدير كمية المركبات النشطة (الفينولات والفلافينويدات) في المستخلص النباتي فكانت mg/g (15.87، 17.33) على التوالي. سجل اختبار (DPPH) وحسب التراكيز المستخدمة mg/ml (20،30،40) نسب تثبيط (21.3، 23.44، 26.24) % على التوالي. كما تم الكشف عن كمية مركبات الاوكسجين الفعالة ROS في المستخلص الجذري حيث اعطت كمية ضئيلة جدا قدرت nml(343). اختبر المستخلص في الخلايا الطبيعية لخط DHSF البشري وبخمس تراكيز µg/ml (25،50،100،200،400) ولثلاث اوقات (24،48،72) ساعة.

النتائج:

سجل أعلى نسبة سمية خلوية عند التركيز µg/ml (400) هي (34.69) % ذلك بعد مرور 72 ساعة من التعرض للمستخلص مع وجود فروق معنوية بين التراكيز العليا عند نسبة احتمال خطا ($P \leq 0.05$) في حين سجلت نسب تثبيط أقل للخلايا عند التراكيز الواطئة أذا أعطت عند اقل التراكيز µg/ml (25) نسبة منخفضة (3.03) %، ولم يلاحظ اي فروق معنوية كبيرة بين التراكيز الواطئة في اوقات التعريض (24.48) ساعة.

الاستنتاجات:

ان المستخلص آمن نسبيا على الخلايا الطبيعية للخط DHSF عند أعلى التراكيز المستخدمة في التجربة اذ بلغ أعلى معدل قتل في الخلايا الطبيعية بعد مرور ثلاث ايام من زمن التعريض .

الكلمات المفتاحية:

نبات الاسل ; DHSF ; مستخلص الهكسان ; DPPH ; سمية خلوية

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