THE EFFECTS OF HABBATUS SAUDA (*NIGELLA SATIVA*) ON CANCER CELL LINES: A SCIENTIFIC AND ISLAMIC APPROACH

Khairina 'Izzati Amir Hussain [a], Assoc. Prof Aminuddin Ruskam [b] & Dr. Salehhuddin Hamdan [c]
[a, b] Faculty of Islamic Civilisation, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.
[c] Faculty of Biosciences and Bioengineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor.
[a] Corresponding author: Phone: +6013-7582616, E-mail: khairinaizzati@gmail.com

Abstract: *Nigella sativa* or black cumin is a Sunnah remedy by the Prophet Muhammad which has been described as a cure for all diseases except death. *Nigella sativa* have shown significant antitumor and cytotoxic activity *in vitro* which may inhibit the growth of cancer cells. This could be considered as an alternative to conventional cancer therapies such as chemotherapy and immunized pig lymphocytes. Studies on the effect of *Nigella sativa* on cancer line have not been widely investigated; therefore, this study is carried out. Cancer cell lines such as HeLa, AsPC-1 Hep-2 and 911 Cells were tested in medium treated with *Nigella sativa* seed extract and oil. After 72 hours incubation, cell viability and the efficacy of *Nigella sativa* to treat cancer cell lines were tested by means of MTT assay through statistical analysis using one-way ANOVA and paired sample T-test. The cell activity will be observed via SEM (*scanning electron microscope).* Hopefully, this study will be beneficial as a preface study for an alternative treatment for cancer.

Abstrak: *Nigella sativa* atau jintan hitam adalah penawar Sunnah oleh Nabi Muhammad yang dikatakan ubat bagi segala penyakit kecuali mati. *Nigella sativa* telah menunjukkan sifat anti-tumor dan aktiviti sitotoksik secara *in vitro* yang boleh menghalang pertumbuhan sel kanser. Ini boleh dianggap sebagai sebuah alternatif untuk terapi konvensional bagi kanser seperti kemoterapi dan limfosit khinzir yang diimunisasi. Kajian mengenai pengaruh *Nigella sativa* pada sel kanser belum diselidiki secara mendalam; oleh itu, kajian ini dijalankan. Sel kanser seperti HeLa, ASPC-1, Hep-2 dan Sel 911 diuji dalam medium yang dirawat dengan ekstrak biji *Nigella sativa* serta minyaknya. Setelah inkubasi selama 72 jam, kelangsungan hidup sel dan keberkesanan *Nigella sativa* untuk mengubati sel kanser diuji dengan cara MTT assay melalui analisis statistik menggunakan ANOVA sehala dan sampel padanan T-test. Aktiviti sel akan diperhatikan dibawah SEM (*scanning electron microscope).* Kajian ini diharapkan akan memberi manfaat sebagai kajian permulaan untuk agen baru dalam rawatan kanser.

Keywords: *Nigella sativa*; cancer cell lines; alternative cancer treatment; MTT-Assay; SEM

1. INTRODUCTION

Cancer cells are cells which has uncontrolled growth through a multi-step process that involves the gradual transformation of a normal cell to a tumor cell. This could be caused by mutation and subsequent inactivation of pathway that act to restrain proliferation and conversely, the activation of those which promote proliferation. Many mutations occur in proto-oncogenes and tumour suppressor genes, resulting in the cancer cell becoming liberated from its division cycle [1]. Cancer has become one of the leading death related disease in the world. In Malaysia, it is estimated that the annual occurrence of cancer is as high as 30 000 patients, out of which, a majority of them are found at late stages of the disease [2].

Efforts to overcome this disease have ranged from conventional treatments such as chemotherapy and radiation to natural traditional remedies. Some of the natural compounds for cancer treatments that can be found in plants are flavopiridol, homoharringtonine, β-lapachone and combretastatin A4 which are respectively obtained from the stems of *Amoora rohituka*, the Chinese tree *Cephalotaxus harringtonia*, bark of the lapacho tree (*Tabebuia avellanedae*) and stem wood of the South Africa tree *Combretum caffreum* [3].

Another plant source of natural cancer treatment can be found in Habbatus Sauda. Habbatus Sauda are black cumin in Arabic and scientifically known as *Nigella sativa*. They have been used for thousands of years as a spice and food preservative, as well as a protective and therapeutic remedy for abundant diseaseses [4]. In Islam, there is a common credence that blackseed is a cure for all ailments, but cannot prevent aging or death. This was based on the Sunnah of Prophet Muhammad which was recorded in the book *Al-Mustadrak alaa al-Sahihain* [5]. It was narrated that Abu Hurairah r.a. related from the Prophet (PBUH) that he said: "Use the Black Seed, because it contains a cure for every type of ailment, except for death." (At-Tirmidh, Ahmad and Ibn Hibban).

Previous studies on *Nigella sativa* have shown significant antimutagenic activity and antioxidant capacity in different assays *in vitro* [6]. It has also shown antimicrobial activity in multi-drug resistant bacteria, antiasmathic effects in airways of asmathic patients and antifungal activity towards a number of phytopathogenic fungi [7, 8, 9].

In treating cancer, *Nigella sativa* has shown cytotoxic effects [10] and several anti-cancer properties such as potent immunomodulators of splenocyte response, Th1 versus Th2 immunereactions, macrophage inflammatory responsiveness, and NK activity against tumor formation and progression [11]. It has also been proven that thymoquinone, an active compound in *Nigella sativa* demonstrated cytoxicity towards SiHa cell line and inducing cell apoptosis [12].

This study is conducted to determine the effect Habbatus Sauda (*Nigella sativa*) on cancer cell lines by using its seeds and oil extract. There are basically four cancer cell lines which are cervical carcinoma (HeLa), breast cancer cells (CRL 2327), human retinoblastoma cells (911 Cells) and laryngeal carcinoma (Hep-2) which will be cultured onto cell medium DMEM, L15 and RPMI. They were tested for viability and cell activity by means of MTT assay and SEM (*scanning electron microscope).*

2. MATERIALS AND METHODS

Cell lines

The cell lines that were used are cervical carcinoma cells (HeLa), breast cancer cells (CRL 2327), human embryonic retinoblasts (HER) 911 cells and human epithelial type-2 (Hep-2).

Cell culture

All the cell lines were cultured in DMEM, RPMI 1640 and L15 medium, each added with 10% Foetal Bovine Serum (FBS), L-glutamine and penicillin-strep.

Cell media

The cell media that were used are L15 medium, DMEM and RPMI 1640 which were obtained from American Type Culture Collection (ATCC) Biological Resource Centre.

Preparation of *Nigella sativa*
Two types of habbatus sauda will be used which are its seeds and oil extract which are obtained from a local herb shop in Makkah, Saudi Arabia.

The seeds were washed with Phosphate-buffered saline (PBS) three times and dried before grounding it in liquid nitrogen until complete evaporation. 10ml ddH_20 were then added to 20g of the grounded seeds and stirred overnight using magnetic stirrer to allow extraction. The crude extract was centrifuged at 10,000 × g for 15 min at room temperature. The supernatants are harvested using rotatory evaporator until evaporation was complete and 20mg/ml stock concentration was prepared and sterilize by filtration using Nalgene 0.22 μm filters. The aqueous extracted from the seeds and the pre-extracted oil bought from Makkah were dissolved in DMSO at a concentration of 10–200 mg and stored at -80 °C.

Treatment of cell culture using *Nigella sativa*
The cells were seeded in a flask at density of 2–4 X 10^4 cells/cm^2 and grown in 12-well plates until confluency is reached. The cells were washed with serum-free medium twice before adding 0.5 ml medium, 0.5 % Fetal Calf Serum (FCS), 0.25 % bovine serum albumin to each well. The cells were incubated overnight in low-serum conditions before stimulation. The solution prepared using *Nigella sativa* was added to the medium in a volume corresponding to 0.1% of the culture.

Measurement of cell proliferation (MTT Assay)
The treated cells were maintained in medium supplemented with 10% Fetal Bovine Serum (FBS) and penicillin-strep. The cells are seeded into 96-microwell plate (200ul/well) and incubated (37C, 5% CO_2) to allow the cells to attach to the wells. After overnight incubation, the medium was replaced with refresh medium containing *Nigella sativa* and incubated for 72 hours (37C, 5% $CO_{2)}$. After 72 hours, the samples were checked for cell viability using MTT assay which detects dehydrogenase activity in viable cells. The old medium was discarded and MTT was added to each well at concentration of 20μl before being incubated for 4 hours at 37C. The precipitates were dissolved in 160μl DMSO. The absorbance of samples was read using ELISA at 570nm.

3. RESULTS AND DISCUSSION

Treatment of 911 cells with *Nigella sativa* seeds and oil

Figure 1.0 shows the inhibition of 911 cells when treated with *Nigella sativa* aqueous seed extract and oil at concentration 50, 100, 150 and 200 mg/mL. The oil shows a higher inhibition compared to the seeds. The highest inhibition can be seen using 200 mg/mL oil which is 70.6% and only 38.2% by seeds. The lowest inhibition was seen in 50 mg/mL of seeds which was only 13.2%.

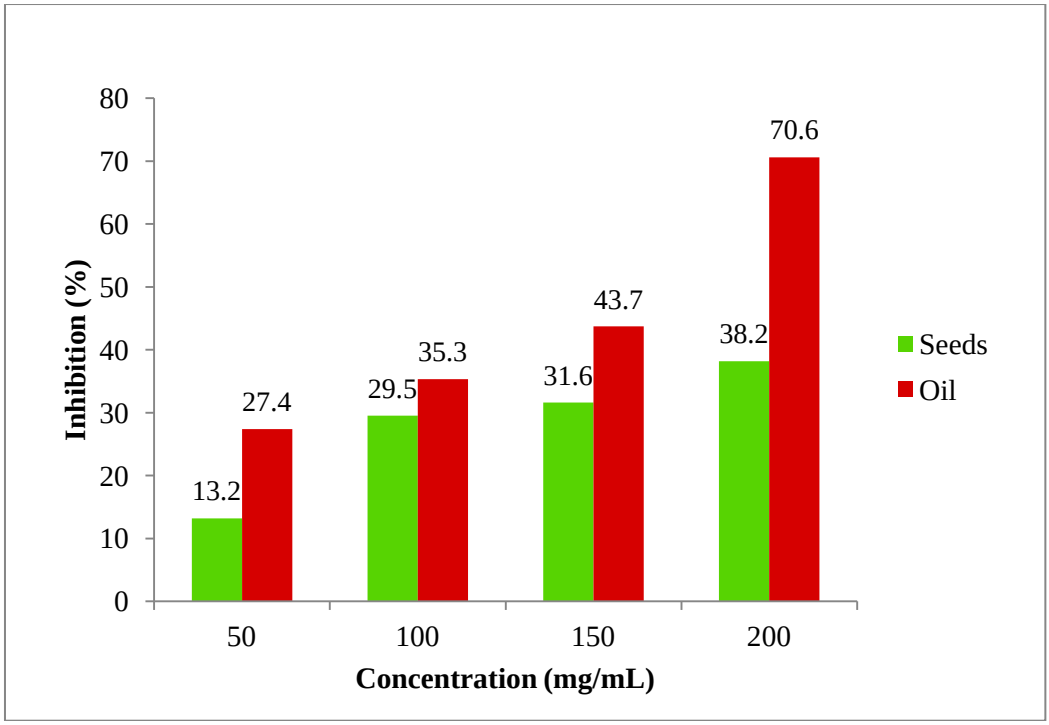

Figure 1.0: Inhibition of 911 cells using *Nigella sativa* aqueous seed extract and oil at different concentrations.

Cytotoxic activity of both aqueous extract of N. sativa seeds and its oil can be seen based on the percentage of inhibition shown in Figure 1.0. This could be explained as thymoquinone and dithymoquinone are active compounds from N. sativa seeds that have shown to be cytotoxic to some parental and multidrug resistant human cancer cell lines [13]. Previous study has shown that thymoquinone induces apoptosis of cancer cell lines by significant elevation of p53 and down-regulation of the anti-apoptotic Bcl-2 protein in the treated cells [11]. Further studies needs to be conducted to confirm whether these compounds are the cause for cytotoxicity of N. sativa when treated on cancer cell lines.

Further studies and conclusion

This preface study will be continued with the treatment of *Nigella sativa* on carcinoma cells (HeLa), breast cancer cells (CRL 2327) and human epithelial type-2 (Hep-2). It is expected that the growth of cancer cell lines will be inhibited once treated with medium containing *Nigella sativa* and apoptosis will eventually occur. Determination of mode of cell death will be investigated by Annexin V/PI staining where the number of viable, early apoptotic, late apoptotic and necrotic cells will be quantified by flowcytometry.

The composition of *Nigella sativa* aqueous seed extract and oil will be analysed to understand the significant difference that occurred after treatment. It will also be related to the sunnah of the Prophet on how to consume *Nigella sativa*.

Since cancer is the one of major health threats for human throughout the world, the treatment of *Nigella sativa* on cancer cell lines *in vitro* is expected to lead the improvement of the pharmaceutical development on overall health status and prolongation of life span in cancer patients as well as an alternative to conventional cancer treatments such as chemotherapy, monoclonal antibodies, radiations, pigs and swines.

REFERENCES

[1] Mclaughlin, F., Finn, P. & La Thangue, N. B. 2003. The Cell Cycle, Chromatin and Cancer Mechanism-Based Therapeutics Come of Age. *Drug Discovery Today*. 8**:** 793-802.

[2] Lim, G. C. C. 2002. Overview of Cancer in Malaysia. *Jpn. J. Clin. Oncol.* 32**:** S37-42.

[3] Nobili, S., Lippi, D., Witort, E., Donnini, M., Bausi, L., Mini, E. & Capaccioli, S. 2009. Natural Compounds for Cancer Treatment and Prevention. *Pharmacological Research*. 59: 365–378.

[4] Chopra, R. N., Nayar S. L. & Chopra I. C. 1956. *Glossary of Indian medicinal plants*. New Delhi: Council of Scientific and Industrial Research.

[5] Ibn Qayyim Al-Jauziyah. 2003. *Healing With The Medicine of The Prophet, Second Edition*. Riyadh: Maktaba Dar-us-Salam.

[6] Bourgou, S., Ksouri, R., Bellila, A., Skandrani, I., Falleh, H. & Marzouk, B. 2008. Phenolic Composition and Biological Activities of Tunisian *Nigella sativa* L. Shoots and Roots. *C. R. Biologies*. 331: 48–55.

[7] Salman, M. T., Khan, R. A. & Shukla, I. 2008. Antimicrobial Activity of *Nigella sativa* Linn. Seed Oil Against Multi-Drug Resistant Bacteria From Clinical Isolates. *Natural Product Radiance, Vol. 7*. 1: 10-14.

[8] Boskabady, M.H., Mohsenpoor, N. & Takaloo, L. 2010. Antiasthmatic Effect of *Nigella sativa* in Airways of Asthmatic Patients. *Phytomedicine*. 17: 707–713.

[9] Rogozhin, E.A., Oshchepkova, Y. I., Odintsova, T. I., Khadeeva, N. V., Veshkurova, O. N., Egorov, T. A., Grishin, E. V. & Salikhov, S. I. 2011. Novel Antifungal Defensins From *Nigella sativa* L. Seeds. *Plant Physiology and Biochemistry*. 49: 131-137.

[10] Thabrew, M.A, Mitry, R. R., Morsy, M. A. & Hughes, R. D. 2005. Cytotoxic Effects of a Decoction of *Nigella sativa*, Hemidesmus indicus and Smilax glabra on Human Hepatoma Hepg2 Cells. *Life Sciences*. 77: 1319–1330.

[11] Majdalawieh, A. F., Hmaidan & Carr R. I. 2010. *Nigella sativa* Modulates Splenocyte Proliferation, Th1/Th2 Cytokine Profile, Macrophage Function and NK Anti-tumor Activity. *Journal of Ethnopharmacology*. 131: 268–275.

[12] Ng, W.K., Yazan, L. S. & Ismail, M. 2011. Thymoquinone from *Nigella sativa* was more potent than cisplatin in eliminating of SiHa cells via apoptosis with down-regulation of Bcl-2 protein. *Toxicology in vitro.*

[13] Worthen, D.R., Ghosheh, O. & Crooks, P.A. 1998. The In-Vitro Anti-Tumour Activity of Some Crude and Purified Compounds of Black Seed, *Nigella sativa*. Anticancer Research. 18: 1527–1532.