



2-1-2022

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### How to Cite This Article

Shaer, Nehad and Al-Abbas, Nouf (2022) "Potential effect of Chia Seeds Crude Extract Nanoparticles on Mcf-7 Breast Cancer Cell Line," *Al-Azhar International Medical Journal*: Vol. 3: Iss. 2, Article 21.

DOI: <https://doi.org/10.21608/aimj.2022.97818.1585>

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## Potential effect of Chia Seeds Crude Extract Nanoparticles on Mcf-7 Breast Cancer Cell Line

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Received for publication September 29, 2021; Accepted February 22, 2022; Published online February 22, 2022.

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doi: 10.21608/aimj.2022.97818.1585

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### ABSTRACT

**Background:** Cancer of breast is one of the most popular causes of cancer-related mortality in women. It is a famous condition among women in Saudi Arabia, with 21.8% incidence. It is rated as the second women cancer and its rate is expected to increase over the next few years.

**Aim of the work:** Focusing on increase the bioavailability of Chia seeds (Chs) and increase its efficiency against breast cancer using nano delivery system.

**Material and Methods:** Chia seeds Poly(lactide-co-glycolide)-Poly(ethylene glycol) (PLGA-PEG)- nanoparticles were prepared and characterized with Transmission Electron Microscope (TEM), Entrapment efficiency measurement and Particle size distribution with zeta potential analyses were carried out. The anti-breast cancer activity was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetry assay and flow cytometry in addition, the Chs nanoparticles were used to assess cell migration in wound healing assay.

**Result:** The TEM image showed spherical NPs with a nano-capsule of PLGA and PEG with Z-average diameters was 62.6 nm. The polydispersity index (PDI) was 0.1 which reflects the high stability of the NPs. Negative charge (zeta potential) showed a consistent pattern on the surface of Chs-loaded PLGA-PEG NPs (ZP= -11.9 mV). Chs NPs exert high cytotoxic effect than Chs. Moreover, the Chs NPs inhibit the cell migration in MCF7 cells. More interestingly that Chs exert apoptosis in breast cancer cells as compared with normal skin fibroblast cells.

**Conclusion:** The present pilot study showed that Chs NPs has anti-cancer activity and highlight its role as complementary therapy.

**Keywords:** Chia Seeds; Mcf-7 Breast Cancer Cell Line; MTT assay; Apoptosis.

**Disclosure:** The authors have no financial interest to declare in relation to the content of this article. The Article Processing Charge was paid for by the authors.

**Authorship:** All authors have a substantial contribution to the article.

### INTRODUCTION

Cancer is a term used to describe disorders in which aberrant cells divide out of control and spread to other parts of the body. Breast cancer is one of the extremely serious and popular cancers in women, and it is also one of the main reasons of mortality with an incidence of 21.8 percent. Breast cancer is a predominant malignancy among Saudi women. It's considered the ninth leading cause of death according to the most recent census of cancer-related mortality.<sup>1-3</sup> According to reports, breast cancer is the second most widespread malignancy in Saudi women, and experts estimate that breast cancer rates is increasing in the coming decades as the population increases as well as advancing ages.<sup>4, 5</sup> The King Faisal Specialist Hospital and Research Centre's Saudi Cancer Registry reports that about 930 new patients of breast cancer are recorded a year. Breast cancer was the most prevalent newly diagnosed cancer among women in Saudi Arabia in 2010,

accounting for 1,473 (27.4%) of the 5,378 cancer diagnoses<sup>6</sup>

Complement therapy has recently gained a lot of attention in cancer prevention and management. Chia seed (Chs) is a good source of omega-3 polyunsaturated fatty acids and it has lipid-lowering, anti-inflammatory, metabolic-regulating and anti-hyperglycaemic properties, which are useful in the managements of chronic diseases, particularly metabolic ones.<sup>7,8</sup> Proteins and phytochemicals are also present in significant amounts. Chia is utilised in the prevention of numerous non-infectious disorders, including obesity, hypertension, cardiovascular diseases (CVDs), cancer, and diabetes, due to its nutritional content.<sup>9</sup> A simple process involving spontaneous emulsification during lipid hydration can be utilised to create stable Nanoliposomes and Nanoemulsions from Chia seed oil and phospholipid-rich residue, which could be valuable in cosmetics, pharmaceuticals, and other health applications.<sup>10</sup> Furthermore, there are currently international

collaborations (Chia-Link International Network) for ongoing research investigating the potential chia seed health benefits, as well as the development of functional foods derived from it, and as part of these studies, we are focusing on increasing the bioavailability of Chs and increasing its efficiency against breast cancer using nano delivery systems.

## MATERIAL AND METHODS

### 1. Preparation of Chia seeds PLGA-PEG nanoparticles

Preparation of Chia seeds PLGA-PEG nanoparticles was performed by using an oil-in-water (O/W) and prepared according to the methods developed by Abd-Rabou and Ahmed.<sup>11</sup> Briefly, 50 mgs of PLGA polymer were added to 1.5 ml of chloroform. Thus, a primary emulsion was formed. This primary emulsion was added to aqueous PVA solution (6 ml, 2% w/v) for further emulsification to form an oil-in-water emulsion. A microtip probe sonicator was used for that purpose (VC 505, Vibracell Sonics, Newton, USA). Solution was set over an ice bath, with an energy output of 55W for 2 minutes. There was a continuous overnight stirring of the emulsion for the sake of completely evaporating the organic material. The excess amounts of PVA were separated at the next day through ultracentrifugation 50,602xg under cooling (4°C) for 20 min (Sorvall Ultra speed Centrifuge, Kendro, USA) then washed thrice with double distilled water. 5% w/w PEG with molecular weight 2 kD was mixed with the PVA aqueous solution prior to emulsification to get PLGA-PEG NPs (Nano-void).

### 2. Characterization with measurement of Entrapment efficiency

Characterization with Entrapment efficiency measurement was measured with the BMG Labtech microplate reader (Germany). The ratio of the amount of compound incorporated into the NPs to the total amount of compound added was calculated and considered as the entrapment efficiency, transmission electron microscopy (TEM, Philips CM-10, FEI Inc., Hillsboro, OR, USA) was used to examine particle morphology and photon correlation spectroscopy technique was used to calculate particle size and zeta potential analyses.

### 3. Measurement cell viability

Cell culture: two cell lines were utilized in our study, WI-38 normal lung fibroblast cells and MCF-7 breast cancer cells were obtained from VACSERA - Cell Culture Unit, Cairo, Egypt. The originally cell lines were obtained from the American Tissue Culture Collection (ATCC) and cultured in RPMI 1640 supplemented with 10% FBS and 1% Pen/strep and incubated in 37°C and 5% CO<sub>2</sub>. Cell cytotoxicity: MTT cell viability assay were used to assess the cytotoxic effect of different concentrations of Chia seeds crude extract (Chs) and Chs Nps on breast cancer cells. MTT assay is a colorimetric assay for assessing cytotoxicity or cytostatic activity. Under defined condition, NADPH dependent cellular oxidoreductase enzymes reflects the number of viable cells. The MTT get in the cells and passes into

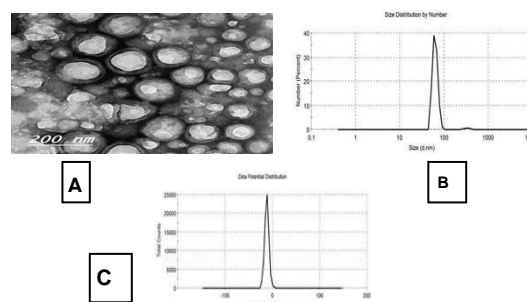
the mitochondria, this enzyme reduce the tetrazolium salt MTT which have purple color, the cells are soluble in organic solvents like DMSO, acidified isopropanol will be the solvents to make the soluble of insoluble formazan. The obtained color will be processed for OD and reads the obtained data<sup>12</sup>. Wound healing assay to assess the migration and proliferation of cancer cells with the different Chs and Chs Nps was as previously described by Noolu *et al*<sup>13</sup>, wounds were made manually using a pipette tip. Cells were washed with PBS and then treated for 48 hours with the extract at the indicated doses.

### 4. Determination of cytotoxicity mechanism

Flow cytometry was used to assess apoptotic and necrotic cell death with Chs and Chs Nps on MCF-7 breast cancer cells and WI-38 normal fibroblast cells, all methods were worked as Al-Abbas and Shaer.<sup>14</sup>

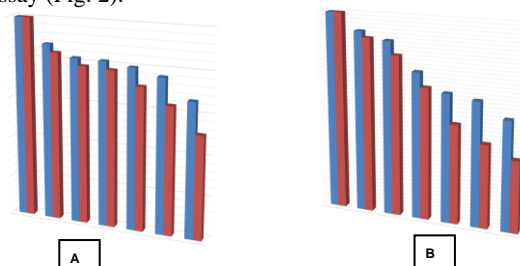
## RESULTS

In the Fig 1, shows TEM image of Chia seeds crude extract nanoparticles spherical NPs with a nanocapsule of PLGA and PEG(A), (B) shows the size distribution of the Ls Nps (62.9 nm) using Zeta Sizer apparatus and (C) shows the Zeta potential of the Ls Nps (ZP= -11.9 mV) using Zeta Sizer apparatus.



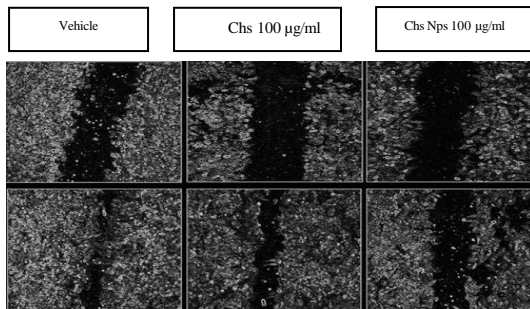
**Fig. 1:** (A) TEM image of Chs Nps, (B) The size distribution of the LsNps and (C) The Zeta potential of the Ls Nps (ZP= -11.9 mV) using Zeta Sizer apparatus.

The effect of different concentrations of Chia seeds crude extract (green columns) and Chia seeds nanoparticles extract (red columns) against of normal cells WI-38 and MCF-7 cells after 24 hrs. of treatment were assessed using MTT cell viability assay (Fig. 2).



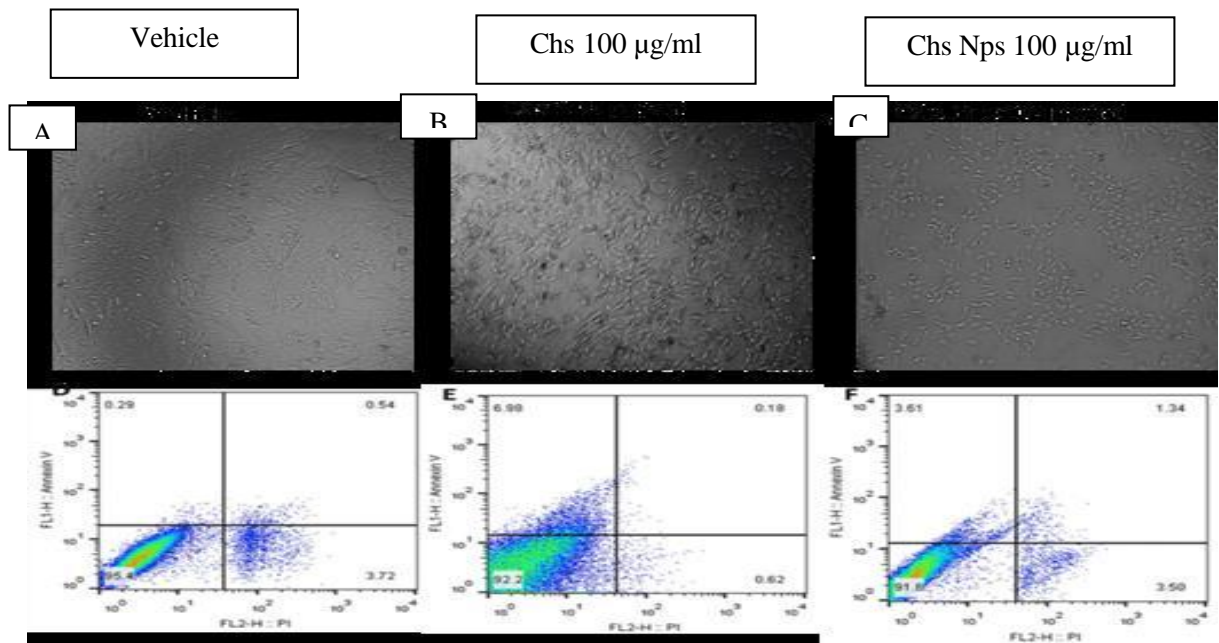
**Fig. 2:** Effect of different concentrations of Chs (blue columns) and Chs Nps (red columns) against of (A) WI-38, (B) MCF-7 cells after 24 hrs. of treatment were assessed using MTT cell viability assay.

Wound healing assay (Fig.3) shows the effect of different preparations (vehicle, Chia seeds crude extract, and Chia seeds nanoparticles extract) on wound healing of the MCF-7 cells.

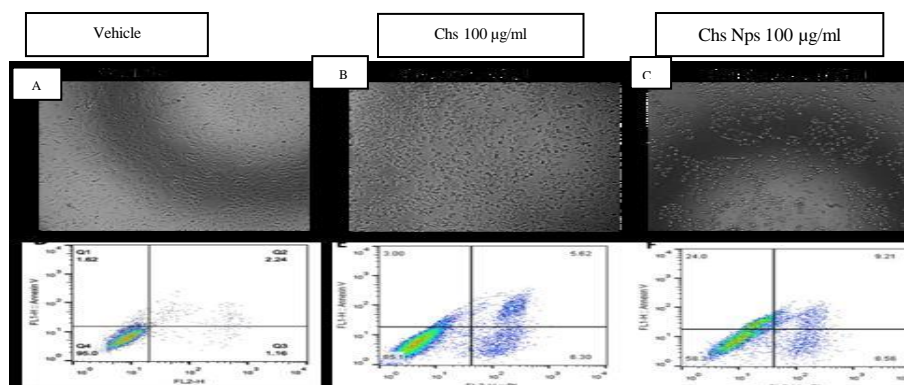


**Fig. 3:** Wound healing assay shows the effect of different preparations (vehicle, Chs extract, and Chs Nps extract) on wound healing of the MCF-7 cells. First row is at 0 hr time point. Second row is 24 hrs after treatment.

MCF-7 cells and WI-38 normal lung fibroblast cells were used for detection of apoptotic and necrotic cell death using Annexin V and Propidium Iodide (PI) staining and their analysis was performed using flow cytometry. (Fig.4, 5) and show that the Chia seeds crude extract nano particles have a significant anti-migration effect on MCF-7 breast cancer cells than the crud extract.



**Fig. 4:** (A-C) bright field microscope images of WI-38 normal lung fibroblast cells. Detection of apoptotic and necrotic cell death using Annexin V and Propidium Iodide (PI) staining and analyzed by flow cytometry. (D) WI-38 vehicle treated cells, (E) WI-38 cell treated with 100 µg/ml Chs and (F) WI-38 cells treated with 100 µg/ml of Chs Nps after 24 Hrs of treatment.



**Fig. 5:** (A-C) bright field microscope images of MCF-7 cells. Detection of Ap optotic and necrotic cell death using Annexin V and Probidium Iodid (PI) staining and analyzed using flow cytometry. (D) MCF-7 vehicle treated cells, (E) MCF-7 cell treated with 100 µg/ml Chs and (F) MCF-7 cells treated with 100 µg/ml of Chs Nps after 24 Hrs. of treatment.

## DISCUSSION

Many authors are now researching the nutritional and medicinal benefits of chia<sup>15, 16</sup>. Chia seeds are rich in polyunsaturated fatty acids as omega-3 and omega-6, as well as soluble dietary fibre. It also has a good amount of phytochemicals and proteins. The chemical makeup, nutritional characteristics, antioxidant and antibacterial activity, as well as the extraction processes utilised to make chia oil, will be covered in detail<sup>17,18</sup>. The latter authors added that Chia seeds are said to have a positive effect on blood lipid profile improvement. Experiments have shown that they have hypotensive, hypoglycaemic, antibacterial, and immunostimulatory properties. Chia is utilised for prevention of numerous non-infectious disorders including hypertension, obesity, cancer, cardiovascular diseases (CVDs), and diabetes due to its nutritional value<sup>19</sup>. Chia seeds looked at cardioprotective effects. A-linolenic acid is involved in the synthesis of several important biochemical molecules, including leukotrienes and thromboxanes, which are linked to a variety of physiological processes in the human body, omega-3 fatty acids can prevent hypertension by inhibiting calcium and sodium channel dysfunctions, increasing parasympathetic tone, and protecting against ventricular arrhythmia., ingesting of chia seeds throughout pregnancy aids in the development of the foetus' retina and brain<sup>20</sup>.

Several studies investigated anti-proliferation activity of Chia Seed Oil against different cancer cells<sup>11,12,17,19</sup>. However, our study is the first to evaluate Chia seeds crude extract in a nanoparticle manner. This study found that Chia seeds crude extract has no cytotoxic effect on the normal cells and the nano particles have an obvious cytotoxic effect on MCF-7 breast cancer cells than the crude extract.

Chia seeds crude extract nano particles have a significant anti- migration effect on MCF-7 breast cancer cells than the crude extract and induce markedly apoptotic cell death on MCF-7 breast cancer comparing with the normal WI-38 cells. Other research has indicated that seed oils such as canola, flaxseed, walnut, neem and squash, hinder the propagation of numerous cancer cell lines<sup>21, 22</sup>. According to a study conducted by Ortega and Campos<sup>23</sup>, breast cancer cells viability could be significantly increased at high concentrations of chia seed oil, however at low doses, it may impair cell viability.

It's vital to remember that the effects of the oil in *vitro* aren't extrapolated to the systemic level; this is due to the possibility of fatty acid breakdown and absorption by other organs prior to approaching the breast tissue. In a model of mammary gland adenocarcinoma in mouse, Espada et al.<sup>24</sup> investigated the effects of *Carthamus tinctorius* and *Salvia hispanica* oil on eicosanoid synthesis, growth, and metastasis. In the present study, the aqueous extract of Chia seeds crude inhibited cell proliferation and induced apoptotic cell death of the

cancer cell lines, Quintal-Bojórquez et al.<sup>25</sup> demonstrated that the present low molecular weight peptides, obtained from chia seeds, as a potential adjuvant option for treatment of cancer.

TEM image of Chs Nps demonstrated the size and distribution of the LsNps and the Zeta potential of the LsNps (ZP= -11.9 mV) using Zeta Sizer apparatus as recommended by Khorsandi *et al.*<sup>26</sup> who declared that the toxicity of breast cancer cells depends mainly on necroptosis and other multiple cell death pathways. Effect of different concentrations of Chs and Chs Nps against WI-38, B) MCF-7 cells after 24 hrs. of treatment were assessed using MTT cell viability assay. In both cancer cell lines, annexin V binding, data suggest that cell death occurred via the apoptotic mechanism. The activity of the 26S proteasome was considerably reduced by CLE treatment in cancer cells but not in normal cells.<sup>13</sup> Flow cytometry was used to evaluate bright field microscope pictures of WI-38 normal lung fibroblast cells and apoptotic and necrotic cell death using Annexin V and Propidium Iodide (PI) staining.<sup>27, 28</sup>. However, co-staining with Propidium Iodide (PI) distinguishes apoptotic cells from necrotic cells, since PI enters necrotic cells but not apoptotic cells. This technique explains how to detect and quantify apoptotic and necrotic cells using Annexin V binding and PI uptake, followed by flow cytometry.

## CONCLUSION

From the current investigation we can conclude that Chia seeds nanoparticles are a promising adjuvant therapy in breast cancer cells. Chia seed extract loaded on PLGA-PEG nano-delivered system increase the bioavailability to cancer cells. More mechanistic studies are required to uncover the anti-cancer effect of Chia seeds.

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