Research Article

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Putative anti-proliferative effect of Indian mustard (*Brassica juncea*) seed and its nano-formulation

https://doi.org/10.1515/gps-2022-8119
received September 23, 2022; accepted January 11, 2023

Abstract: Over the past few decades, nanotechnology has shown promising prospects in biomedicine and has a proven impact on enhancing therapeutics by facilitating drug delivery. The present study brings an amalgamation of nanoscience and "clean technology" by fabricating nature-friendly nanoparticles (NPs) sans the use of chemical surfactants using Indian mustard seed, *Brassica juncea* L. The as-synthesized NPs were characterized to assess their average size, crystallinity, morphology, and constituent functional groups through conventional techniques: dynamic light scattering (DLS), X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR). The NPs were crystalline in nature and exhibited a mean size of 205.5 nm (PDI of 0.437) being primarily polygonal in shape. Additionally, the therapeutic efficacy of the green NPs was evaluated based on their cytotoxic effect against two human cancer lines, MCF-7 and HepG-2. Both the NPs and the bulk seeds showed a dose-dependent cytotoxic effect. However, an assessment of the antiproliferative/cytotoxic potential of the green NPs *versus* the bulk seeds showed that the NPs were relatively more efficacious on both cell lines. Taken together, the mustard seed NPs could be potential nutraceuticals considering the green credential in their mode of biosynthesis.

Keywords: green synthesis, nanoformulation, Indian mustard seed, *Brassica juncea* L., anti-proliferative effect

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1 Introduction

Plant-based pharmaceuticals are the new age trend in research in biotechnology and medicine. It is an innovative and sustainable approach in therapeutics to combat a host of civilization diseases in the community including cancer. Cancer is commonly characterized by unregulated proliferation of cells. Breast cancer is the second leading cause of mortality among women [1] in both developed and developing regions, with a higher rate in developing countries compared to developed regions [2]. However, there has been significant improvement in the 5-year survival rate in hormone receptor-positive breast cancer [3], cancer recurrence, and subsequent drug resistance seem inevitable [4]. This warrants an imperative need to develop alternative, safer, and natural therapeutic strategies. Furthermore, hepatocellular carcinoma (HCC) is a common type of liver cancer which is also the third leading cause of cancer-related mortalities worldwide, affecting over 500,000 people, and being highly prevalent in Asia and Africa [5]. Epidemiological studies have shown an optimistic association between the consumption of plant-based foods and reduced prevalence of cancer, heart disease, and other degenerative diseases [6,7]. *Brassica juncea* L. belonging to the Brassicaceae family, popularly known as the Indian mustard, has

been known for both its culinary and therapeutic uses since ancient times. Mustard seeds have a strong antioxidant status which is characterized by the presence of bioactive constituents primarily the phenolic compounds attributed to their pharmacological potential [8]. The presence of sulfur-containing compounds such as glucosinolates in cruciferous vegetables such as Indian mustard reduces the risk of various types of cancer, including colon, kidney, and prostate cancer [9,10]. The glucosinolates are hydrolyzed by the enzyme myrosinase present in cruciferous vegetables to yield biologically active isothiocyanates [11]. Isothiocyanates, a phytonutrient that has been widely studied for its anticancer benefits, are important phytocompounds in mustard seeds. Isothiocyanates can inhibit mitosis and stimulate apoptosis (cell death) in human tumor cells and significantly inhibit bladder cancer growth [11]. In addition, in a study on human colon cancer cell lines, it was reported to induce programmed cell death of colon cancer [12].

The present trend in therapeutics encourages the use of alternative and complementary medicine in cancer prevention. Natural products offer an enormously promising approach for chemoprevention to slow down the progression of cancer. Accordingly, several natural phytocompounds/plants have provided modern medicine with the drugs used as cytostatics [13]. It has been reported earlier that the use of phytocompounds in therapy is restricted owing to their low solubility and availability in the biological milieu [14]. The term nanomedicine is commonly used to describe the integrated discipline of science and technology used in prevention, diagnosis, and therapeutics using nanoscale materials carefully designed to perform these functions [15]. Nanomaterials have distinctive physicochemical properties that confer them with an increased specific surface area and enhanced optical, magnetic, or mechanical properties [16]. Thus, carrier systems based on nanoparticles ensure a sustainable release of bioactive substances contributing to an enhanced bioavailability [3]. There is a plethora of literature that reports the phyto-mediated synthesis of several nanoparticles. However, most of the studies mention the use of biogenic synthesis of metallic nanoparticles using plant products/compounds [17]. There is still a paucity of studies based on the biogenic synthesis of non-metallic nanostructures. Keeping the premise, the present study aimed at a phyto-mediated synthesis of nanoparticles without the use of chemical surfactants/reductants with clean technology as the cornerstone. To the best of our knowledge, this is the first study assessing the role of non-metallic mustard seed nanoparticles for which the patent has been granted by USPTO (US) [18].

2 Materials and methods

2.1 Synthesis of nanoparticles

Mustard seeds procured from the local market were washed in tap water and then air-dried. The dried seeds were ground using a mechanical grinder. Mustard seed powder (400 mg) was mixed with 30 mL of solvent with constant stirring; this solution was then sprayed into boiling water (50 mL) at a flow rate of 0.2 mL·min^{-1} for 5 min under ultrasonic conditions (750 W and a frequency of 20 kHz). After 5 min of sonication, the contents were centrifuged at 200–800 rpm for about 20 min at room temperature and dried to obtain mustard seed nanoparticles.

2.2 Characterization of nanoparticles

The synthesized Indian mustard seed nanoparticles were characterized using the following techniques: zetasizer (Nano series, HT-laser, ZEN3600 from Molvern Instrument, UK) was used to analyze the mean size of the mustard seed powder nanoparticles. The scanning electron microscope (SEM) (JEOL JSM-7600F, USA) is one of the most effective electron microscopy techniques that allows accurate assessment of the size, shape, distribution, spatial resolution, and variations in composition and structure of NPs. Mustard NPs were characterized by Fourier transform infrared (FTIR) spectroscopy (Perkin-Elmer FTIR spectrum BX, USA) by mixing nanoparticle dry powder with potassium bromide (KBr). The spectra were recorded in the range from 4,400 to 400 cm^{-1}. FTIR data revealed details of the functional groups in the mustard seeds and nanoparticles. The method for determining the crystal structure or crystal phase is X-ray diffraction. Dried mustard powder was used for analysis. Diffraction patterns were recorded by PAN Analytical XPert PRO (Netherlands) operated at 40 mA and 45 kV using CuK radiation (0.15406 nm). The crystallographic information was recorded in a range from 0° to 100°.

2.3 Evaluation of cytotoxic effects

The potential anti-proliferative effect of the nanoparticles was tested in vitro on MCF-7 cells and HepG-2 cell lines obtained from VACSERA Tissue Culture Unit (Cairo, Egypt). The cytotoxic activity of the nanoparticles was analyzed by 3-(4,5-dimethylthazolk-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetry assay [19]. For the assay, cells were seeded in a 96-well plate at a concentration of 110 × 4 cells·well^{-1} in

100 µL of growth medium. Incubation was allowed for 24 h at 37°C, and then, the cells were allowed to settle. Various concentrations of the mustard seed powder and its nano-formulation were added and incubated for a further 48 h, and the yield of viable cells was colorimetrically determined. All experiments were performed in triplicates. The 50% inhibitory concentration (IC_{50}) was computed from dose-response curve plots using Graphpad Prism software (San Diego, CA, USA).

3 Results

3.1 Characterization of nanoparticles

Particle size distribution for synthesized mustard seed nanoparticles was determined by the DLS technique shown in Figure 1. The sample of synthesized NPs was of variable size, two broad peaks were observed with a higher intensity toward the larger particles, with an average size of 205.5 nm and a PDI of 0.437.

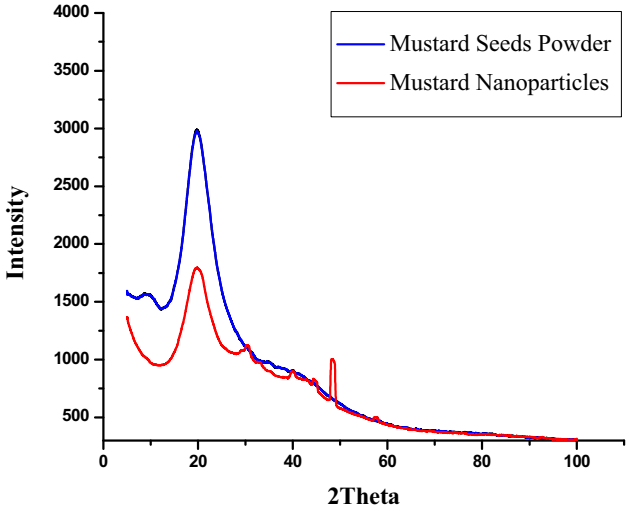

Figure 1: X-ray diffractograms of mustard seeds and mustard nanoparticles.

X-Ray diffractogram of mustard seed powder showed a characteristic high peak at a diffraction angle of 2θ at 22°. A similar peak at a lower intensity appeared for the nano-formulation of the mustard seed that confirms the presence of the crystals of nano mustard. However, in addition to the main peak, which was sharp, three short peaks appeared for the nano mustard at $2\theta \approx 30°$, 40°, and 50° (Figure 2). Therefore, it can be concluded that the nano-fabricated sample was crystallized.

Scanning electron microscope (SEM) images shown in Figure 3 demonstrate the variable shape and heterogeneity in the particle size distribution of mustard seed nanoparticles. The results on the structure of mustard NPs characterized by SEM are in consensus with the DLS analysis. Also, SEM images fairly represent the particles in suspension, showing the shape and size distribution of the synthesized nanoparticles. This exhibited polydispersed nature of the particles corresponding with DLS results. The morphotype of the synthesized nanoparticles is shown in Figure 3a–d, which is seen primarily as polygonal shaped.

FTIR spectroscopy was performed to classify the chemical essence of both the raw mustard seeds and their nano-formulation. Figure 4a and b show the FTIR spectrum of the mustard seeds or mustard nanoparticles in the range of 400–4,000 cm^{-1}. Figure 4b shows a strong absorption band at 3,428.60 cm^{-1} resulting from the stretching of the NH amino band or indicative of the hydroxyl-bonded OH band reflecting the presence of alcohols, phenols, and carbohydrates. The band (2,928.4 cm^{-1}) relates to the asymmetric stretching of the C–H bonds.

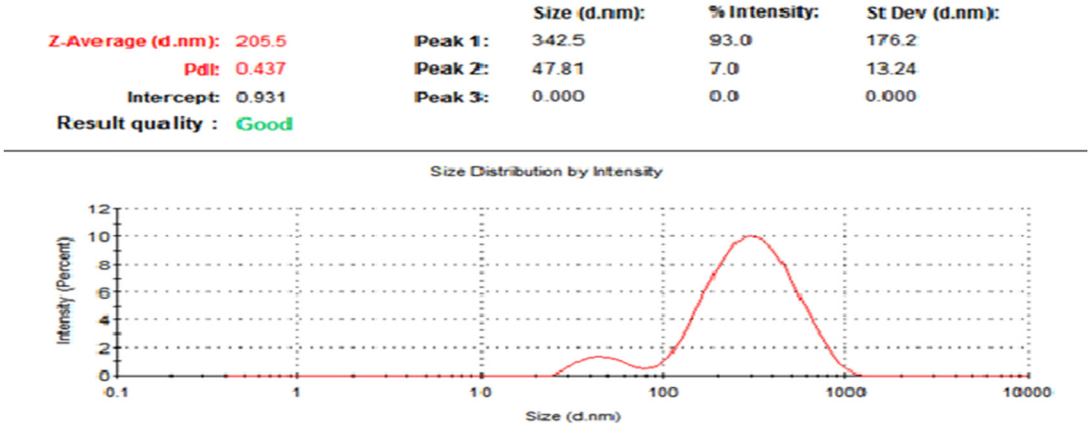

Figure 2: DLS spectrum of mustard seed nanoparticles.

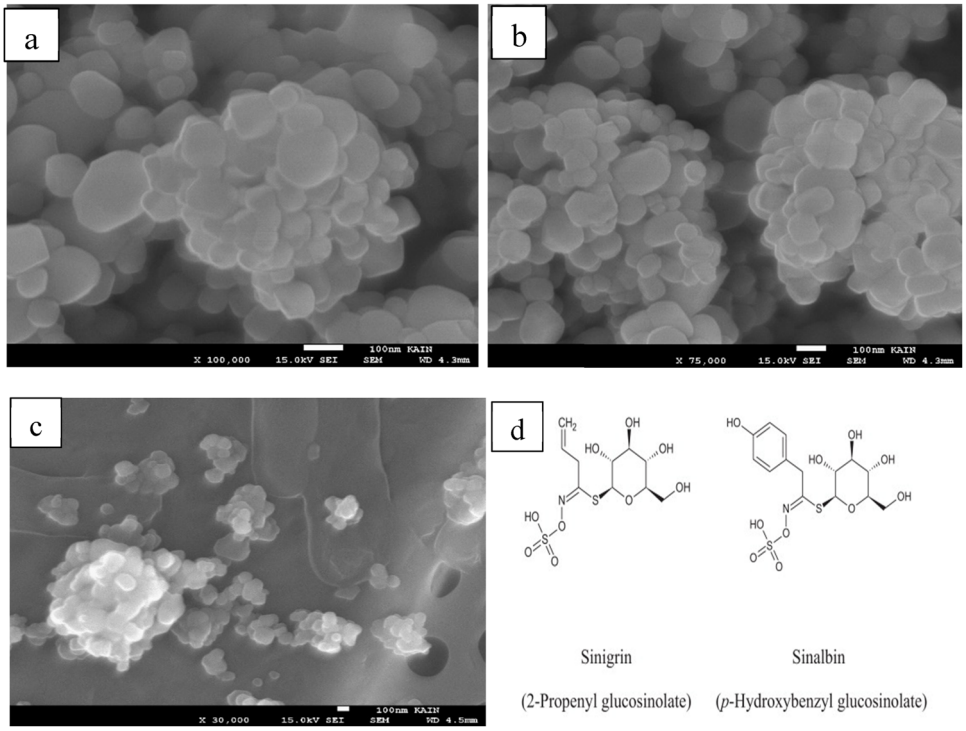

Figure 3: (a–c) Micrographs obtained by scanning electron microscopy, showing morphology and size of the mustard seed nanoparticles. (d) Chemical structure of mustard seed glucosinolates, sinalbin, and sinigrin.

The band at 1,745.48 cm^{-1} shows the fingerprinting region of CO, C–O, and O–H groups, and the bands at 1,657.25, 1,545.48, and 1,422.5 cm^{-1} for aliphatic amines. The absorption band for native proteins at 1657.25 cm^{-1} is similar to that recorded [19]. The band at 1,657.25 cm^{-1} is known as amide I and amide II, which arise from carbonyl (C═O) and amine (NH) stretches in the amide bonds of the protein. Shearing methylene vibrations of proteins could be related to the absorption band at 1,422.5 cm^{-1}. The strong band at 1,103.92 cm^{-1} is recognized as the C–N stretching vibrations of aliphatic amines. The C–H stretch is assigned the band at 719.59 cm^{-1}, and the band at 1,422.5 cm^{-1} corresponds to the C–C stretches for the aromatic ring.

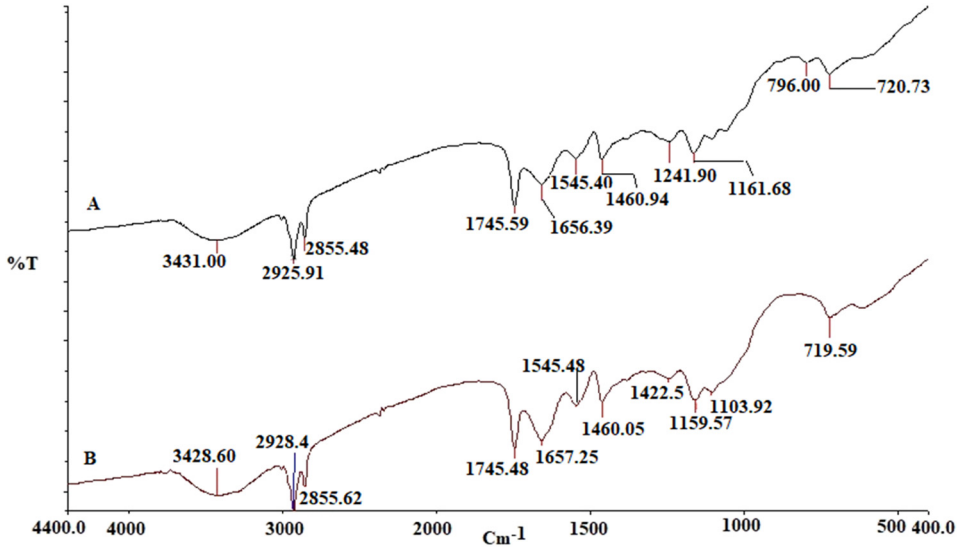

Figure 4: FTIR analysis of mustard seeds (A) and mustard seed nanoparticles (B).

3.2 Anti-cancer activity

The cytotoxic outcome of the mustard seed nanoparticles on human cancer cell lines (MCF-7 and HepG-2) showed a more profound effect than the mustard seed powder. At a high concentration (1,000 μg·mL^{-1}), the mustard seed nanoparticles had an inhibitory percentage of 82.67 on HepG-2 cell growth, while the mustard seed powder showed an inhibitory percentage of 65.94 at the same concentration (Figure 5). For the nanoparticles, the inhibitory effect on HepG-2 cell growth was detectable at a lower concentration of 7.8 μg·mL^{-1}, while for the mustard seed powder, it was observed at 31.25 μg·mL^{-1}, which was 4-fold more than the initial inhibitory concentration of the nanoparticles (Table 1). Additionally, the IC_{50} of the nanoparticles against Hep2-G cells was 477 ± 32.68 μg·mL^{-1}, while the IC_{50} of the mustard seed powder was only 167 ± 16.79 μg·mL^{-1}.

For the MCF-7 cells, the mustard seed nanoparticles produced an inhibitory percentage at the highest tested concentration (1,000 μg·mL^{-1}) of 75.09, while the mustard seed powder at the same test concentration produced an inhibitory percentage of only 55.97 (Table 2 and Figure 6). Further, for the nanoparticles, an inhibitory effect on the MCF-7 cells was detectable at a lower concentration of 15.6 μg·mL^{-1} while for the mustard seed powder, the inhibitory effect was first observed at 62.5 μg·mL^{-1} as shown in Table 2. In addition, the IC_{50} of the mustard seed powder against MCF-7 cells was 781.1 ± 62.44 μg·mL^{-1}, while the IC_{50} of the nanoparticles against MCF-7 cells was only 299.7 ± 60.3 μg·mL^{-1}.

4 Discussion

The current study reported the biogenic synthesis of non-metallic nanoparticles of mustard seed powder on the

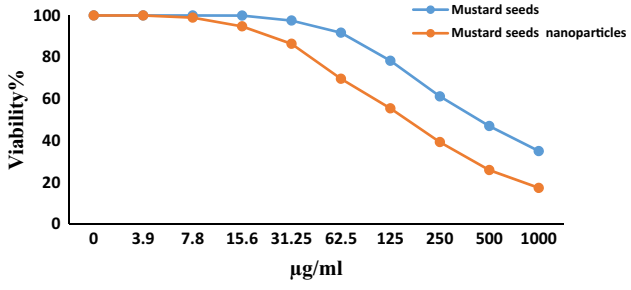

Figure 5: Evaluation of cytotoxicity of mustard seed and its nano-formulation against HepG-2.

Table 1: Inhibitory activity of mustard seed (part A) and the nanoparticles (part B) against HepG-2 cell line

A Concentration (μg·mL^{-1})	Viability % (3 replicates)			Mean	Inhibitory %	S.D.	B Concentration (μg·mL^{-1})	Viability % (3 replicates)			Mean	Inhibitory %	S.D.
	1st	2nd	3rd					1st	2nd	3rd			
1,000	38.91	32.74	30.52	34.06	65.94	4.35	1,000	17.82	14.97	19.21	17.3	82.67	2.16
500	49.23	46.56	45.17	46.99	53.01	2.06	500	26.9	23.81	26.9	25.9	74.13	1.78
250	64.37	60.98	58.25	61.2	38.8	3.07	250	37.23	40.72	39.88	39.3	60.72	1.82
125	81.65	79.82	73.46	78.31	21.69	4.3	125	52.06	56.94	57.31	55.4	44.56	2.93
62.5	92.41	97.63	85.21	91.75	8.25	6.24	62.5	67.34	72.18	69. 4	69.6	30.36	2.43
31.25	98.62	99.87	94.25	97.58	2.42	2.95	31.25	85.62	89.41	84.23	86.4	13.58	2.68
15.6	100	100	99.87	99.96	0.04	0.08	15.6	95.21	96.8	92.37	94.8	5.21	2.24
7.8	100	100	100	100	0	0	7.8	99.76	99.76	97.43	99	1.02	1.35
3.9	100	100	100	100	0	0	3.9	100	100	100	100	0	0
2	100	100	100	100	0	0	2	100	100	100	100	0	0
0	100	100	100	100	0	0	0	100	100	100	100	0	0

Table 2: Inhibitory activity of Mustard seed (part A) and the nanoparticles (part B) against HepG-2 cell line

A Concentration ($\mu g \cdot mL^{-1}$)	Viability % (3 replicates)			Mean	Inhibitory %	S.D.	B Concentration ($\mu g \cdot mL^{-1}$)	Viability % (3 replicates)			Mean	Inhibitory %	S.D.
	1st	2nd	3rd					1st	2nd	3rd			
1,000	46.84	44.37	40.89	44.03	55.97	2.99	1,000	25.47	21.89	27.36	24.91	75.09	2.78
500	59.2	56.43	57.38	57.67	42.33	1.41	500	40.89	36.56	40.89	39.45	60.55	2.5
250	76.43	76.43	79.41	77.42	22.58	1.72	250	53.42	48.17	56.27	52.62	47.38	4.11
125	89.51	87.84	91.37	89.57	10.43	1.77	125	70.84	65.92	71.43	69.4	30.6	3.03
62.5	97.42	98.68	99.53	98.54	1.46	1.06	62.5	89.51	87.24	87.24	88	12	1.31
31.25	100	100	100	100	0	0	31.25	97.26	95.31	96.86	96.48	3.52	1.03
15.6	100	100	100	100	0	0	15.6	100	98.64	99.72	99.45	0.55	0.72
7.8	100	100	100	100	0	0	7.8	100	100	100	100	0	0
3.9	100	100	100	100	0	0	3.9	100	100	100	100	0	0
2	100	100	100	100	0	0	2	100	100	100	100	0	0
0	100	100	100	100	0	0	0	100	100	100	100	0	0

lines of green credentials. The formation of nanoparticles was confirmed by the extensive characterization employed, and the SEM images ensured that the synthesized nanoparticles were within nanoscale size. The DLS results indicated a size of 205.1 for the nanoparticles produced. It has been postulated that DLS measurement is more sensitive to larger particles. These results suggest the calculation of DLS for polydispersed samples may not be accurate [20]. The broadening of the XRD peaks suggested the presence of nanosized crystals in the nanoparticle sample. Broadening of the peaks is usually due to the small crystallite size, and it might be associated with strain (micro deformation) which can be best explained based on the Debye–Scherrer equation [21]. The morphotype of the nanoparticles which was primarily observed as polygonal mirrored the chemical structure of glucosinolates in the mustard seeds, sinalbin, and sinigrin [22]. These glucosinolates are the key constituents that exhibit anti-cancer activity. The FTIR spectra of the bulk and nanosized mustard seeds were quite similar and suggest that the nanosization did not alter the molecular structure of the bioconstituents conferring chemical stability to the nanoparticles. The absorption band for native proteins at 1,657.25 cm^{-1} in the mustard seed was similar to that reported earlier [23]. The band at 3,428.60 cm^{-1} which corresponded to the stretching of the –NH amino/–OH hydroxyl bond group is attributed to the presence of alcohols, phenols, and carbohydrates [24]. FTIR results clearly indicated the presence of groups of carboxyl (–C═O), hydroxyl (–OH), and amine (N–H) in mustard seed and the nanoparticles, which has been documented in the previous literature [25].

In the current study, the antiproliferative/cytotoxic activity of the synthesized nanoparticles was evaluated using the MTT assay. Compared to the bulk seeds, the nanoparticles were more showed a more profound antiproliferative effect on both human cancer cell lines used (HepG-2 and MCF-7). Similar results have been reported

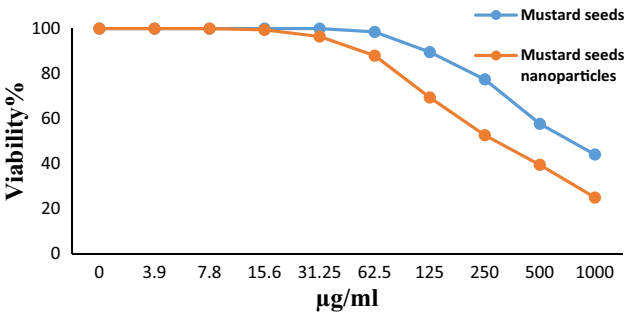

Figure 6: Evaluation of cytotoxicity of mustard seed and its nano formulation against MCF-7.

on the potency of nano-sized plant extracts compared to the bulk material [26,27]. In addition, Abel et al. [26] reported that the silver nano-formulated *Senna tora* leaf extracts had a significant inhibitory effect on colon cancer cells, rather than the leaf extract alone. A mixture of *Olea europaea* fruit extract and *Acacia nilotica* peel extract in gold nanoparticles showed profound anticancer activity against different cancer cell lines (HepG-2, HCT-116, and MCF-7) [24]. In addition, experimental results of a previous study by Hasan et al. [28] demonstrated that mustard seed possesses potent antioxidant-inflammatory effects, and its nanostructure (silver nanoparticles of a mustard seed) was more effective than usual at alleviating oxidative stress, reducing pro-inflammatory cytokines production, and reversing DNA genotoxicity in animal models. The anti-cancer potential of mustard seeds is attributed to the phytochemical profile which reveals the presence phytocompounds: phenols, flavonoids, tannins, and vitamins responsible for their antioxidant status [7,29,30]. It has been recognized that oxidative stress is one of the key players driving the pathogenesis of several diseases including cancer. Indian mustard seeds exhibit potent antioxidant potential that prevents induced carcinogenesis, in consensus with the results of the present study on the anti-cancer activity of mustard seeds, showing that mustard seed powder suppresses the growth of bladder cancer cells *in vitro* and *in vivo* [7]. In addition, mustard seed extract significantly reduced oxidative stress in HepG2 cell line by suppressing reactive oxygen species (ROS) and showed a marked hepatoprotective effect [29]. The vitamin E, quercetin, and catechin detected in the mustard seed extracts also showed hepatoprotective activity in the HepG2 cell line [29]. In addition, allyl isothiocyanate (AITC; 3-isothiocyanato-1-propene or 2-propenyl isothiocyanate) also is a potent anti-cancerous compound that is abundant in cruciferous vegetables such as Indian mustard. Taken together, a synergistic effect of all the phytoconstituents of the mustard seeds contributed to the marked anti-cancer effect of the mustard seed powder and its nano formulation in the present study. The nanosization strategy used in the present study enhanced the bioavailability and thus the efficacy of the mustard seeds. Furthermore, the biosynthetic method was facile, environmentally benign, and cost-effective. Previously, in congruence to the biogenic mode of synthesis employed in the present study, there have been studies on the nanoarchitecture of non-metallic nanoparticles of phytocompounds such as curcumin and naringenin [31–34]. The findings of the present study are further supported by the results of our recent *in vivo* study on the efficacy of synthesized nanoparticles of mustard seed against arsenic toxicity. The results clearly demonstrated a more efficacious attenuating effect of the nanoparticles *versus* the bulk or naïve mustard seeds against arsenic-induced oxidative damage and genotoxicity in a rat model [28,35] supporting the cytotoxic effect of the NPs on the cancer cell lines.

5 Conclusions

In conclusion, the present results demonstrate that the nano-formulation of mustard seeds significantly reduced the cell viability and transformed the cellular morphology of MCF-7 and HepG-2 cells at different concentrations in a dose-dependent manner. However, further *in vivo* and *in vitro* studies at the molecular level are imperative to understand the mechanism(s) of action. The study provides insights into the prospective role of Indian mustard seeds and their nano-formulation in potential adjunct therapy for cancer and as a nutraceutical.

Funding information: This work was funded by the Deanship of Scientific Research at Princess Nourah bint Abdulrahman University, through the Research Groups Program Grant no. RGP-1443-0047.

Author contributions: Promy Virk, Manal A. Awad: conceptualization, writing, supervision; Meznah M. Alanazi, Awatif A. Hendi: data curation, methodology, resource acquisition; Mai Elobeid: data curation, reviewing, editing; Khalid M. Ortashi: visualization, software; Albandari W. Alrowaily, Taghreed Bahlool: investigation, validation; Fatma Aouaini: methodology, reviewing.

Conflict of interest: The authors state no conflict of interest.

References

[1] American Cancer Society. What are the key statistics about breast cancer? http://www.cancer.org/cancer/breastcancer/detailedguide/breastcancer-key-statistics. Accessed: February 4, 2015.

[2] Ferlay J, Parkin D, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer. 2010;46(4):685–850. doi: 10.1016/j.ejca.2009.12.014.

[3] Giessrigl B, Schmidt WM, Kalipciyan M, Jeitler M, Bilban M, Gollinger M, et al. Fulvestrant induces resistance by modulating GPER and CDK6 expression: implication of methyltransferases, deacetylases and the hSWI/SNF chromatin remodeling complex. Br J Cancer. 2013;109:2751–62. doi: 10.1038/bjc.2013.583.

[4] Marquette C, Nabell L. Chemotherapy-resistant metastatic breast cancer. Curr Treat Options Oncol. 2012;13(2):263–75. doi: 10.1007/s11864-012-0184-6.
[5] Rawla P, Sunkara T, Muralidharan P, Raj J. Update in global trends and aetiology of hepatocellular carcinoma. Contemp Oncol (Pozn)/Współczesna Onkologia. 2018;22(3):141–50. doi: 10.5114/wo.2018.78941.
[6] Czapski J. Cancer preventing properties of cruciferous vegetables. J Fruit Ornam Plant Res. 2009;70:5–18. doi: 10.2478/v10032-009-0001-3.
[7] Kaur C, Kapoor H. Anti-oxidants in fruits and vegetables- the millennium's health. Int J Food Sci. 2001;36:703–25. doi: 10.1111/j.1365-2621.2001.00513.x.
[8] Das G, Tantengco OAG, Tundis R, Robles JAH, Loizzo MR, Shin HS, et al. Glucosinolates and Omega-3 fatty acids from mustard seeds: Phytochemistry and pharmacology. Plants. 2022;11(17):2290. doi: 10.3390/plants11172290.
[9] Bhattacharya A, Li Y, Wade KL, Paonessa JD, Fahey JW, Zhang Y. Allyl isothiocyanate-rich mustard seed powder inhibits bladder cancer growth and muscle invasion. Carcinogenesis. 2010;31(12):2105–10. doi: 10.1093/carcin/bgq202.
[10] Bennett RN, Rosa EA, Mellon FA, Kroon PA. Ontogenic profiling of glucosinolates, flavonoids and other secondary metabolites in Eruca sativa (salad rocket), Diplotaxis erucoides (wall rocket), Diplotaxis tenuifolia (wild rocket) and Bunias orientalis (Turkish rocket). J Agric Food Chem. 2006;54:4005–15. doi: 10.1021/jf052756t.
[11] Ahmed AG, Hussein UK, Ahmed AE, Kim KM, Mahmoud HM, Hammouda O, et al. Mustard seed (*Brassica nigra*) extract exhibits antiproliferative effect against human lung cancer cells through differential regulation of apoptosis, cell cycle, migration, and invasion. Molecules. 2020;25(9):2069. doi: 10.3390/molecules2509206.
[12] Grygier A. Mustard seeds as a bioactive component of food. Food Rev Int. 2022;1–14. doi: 10.1080/87559129.2021.2015774.
[13] Ma L, Zhang M, Zhao R, Wang D, Ma Y, Ai L. Plant natural products: Promising resources for cancer chemoprevention. Molecules. 2021;26(4):933. doi: 10.3390/molecules26040933.
[14] Asiri A, Elobeid M, Virk P, Awad M. Ameliorative effect of resveratrol and its nano-formulation on estrogenicity and apoptosis induced by low dose of zearalenone in male Wistar rats. J Mater Res. 2021;36:4329–43. doi: 10.1557/s43578-021-00425-w.
[15] Riyadh M, Gomha S, Mahmmoud E, Elaasser M. Synthesis and Anticancer activities of Thiazoles, 1, 3-Thiazines, and Thiazolidine using chitosan-grafted-poly (vinylpyridine) as basic catalyst. Heterocycles. 2015;91(6):1227–43. doi: 10.3987/COM-15-13210.
[16] Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arab J Chem. 2019;12(7):908–31. doi: 10.1016/j.arabjc.2017.05.011.
[17] Fierascu I, Fierascu IC, Brazdis RI, Baroi AM, Fistos T, Fierascu RC. Phytosynthesized metallic nanoparticles—between nanomedicine and toxicology. A brief review of 2019's findings. Materials. 2020;13(3):574. doi: 10.3390/ma13030574.
[18] Awad MAG, Virk P, Qindeel R, Ortashi KMO, Elobeid MA. Synthesis of mustard seed nanoparticles, US Pat., 10398744, 2019.
[19] Yusuf H, Fahriani M, Murzalina C. Anti proliferative and apoptotic effect of soluble ethyl acetate partition from ethanol extract of *Chromolaena odorata* linn leaves against hela cervical cancer cell line. Asian Pac J Cancer Prev. 2022;23(1):183–9. doi: 10.31557/APJCP.2022.23.1.183.
[20] Domingos R, Baalousha M, Ju-Nam Y, Reid N, Tufenkji N, Lead J, et al. Characterizing manufactured nanoparticles in the Environment: Multimethod determination of particle sizes. Env Sci Technol. 2009;43(19):7277–84. doi: 10.1021/es900249m.
[21] Li B, Jiang L, Li X, Ran P, Zuo P, Wang A, et al. Preparation of Monolayer MoS2 quantum dots using temporally shaped femtosecond laser ablation of bulk MoS2 targets in water. Sci Rep. 2017;7(1):11182. doi: 10.1038/s41598-017-10632-3.
[22] Popova I, Morra M. Sinigrin and sinalbin quantification in mustard seed using high performance liquid chromatography–time-of-flight mass spectrometry. J Food Compos Anal. 2014;35(2):120–6. doi: 10.1016/j.jfca.2014.04.011.
[23] Yilmaz M, Mehmet A, Turkdemir H, Bayram E, Çiçek A, Mete A, et al. Biosynthesis of silver nanoparticles using leaves of *Stevia rebaudiana*. Mater Chem Phys. 2011;130(3):1195–202. doi: 10.1016/j.matchemphys.2011.08.068.
[24] Akhlagh H. Green synthesis of silver nanoparticles using *Pimpinella anisum* L. seed aqueous extract and its antioxidant activity. J Chem Health Risks. 2015;5:257–65.
[25] Khatami M, Soltani N, Pourseyed S. Biogenic synthesis of silver nanoparticles using mustard and its characterization. Int J Nanosci Nanotechnol. 2016;11(4):281–8.
[26] Abel E, Poonga J, Panicker G. Characterization and in vitro studies on anticancer, antioxidant activity against colon cancer cell line of gold nanoparticles capped with *Cassia tora* SM leaf. Appl Nanosci. 2016;6:121–9. doi: 10.1007/s13204-015-0422-x.
[27] Awad A, Eisa N, Virk P, Hendi A, Ortashi K, Mahgoub A, et al. Green synthesis of gold nanoparticles: preparation, characterization, cytotoxicity, and anti-bacterial activities. Mater Lett. 2019;256:126608. doi: 10.1016/j.matlet.2019.126608.
[28] Hassan SA, Hagrassi AME, Hammam O, Soliman AM, Ezzeldin E, Aziz WM. Brassica juncea L. (Mustard) extract silver nanoparticles and knocking off oxidative stress, proinflammatory cytokine and reverse DNA genotoxicity. Biomolecules. 2020;10(12):1650. Published 2020 Dec 9. doi: 10.3390/biom10121650.
[29] Parikh H, Pandita N, Khanna A. Phytoextract of Indian mustard seeds acts by suppressing the generation of ROS against acetaminophen-induced hepatotoxicity in HepG2 cells. Pharm Biol. 2015;53(7):975–84. doi: 10.3109/13880209.2014.950675.
[30] AlDiwan M, ALWahhap Z, AlMasoudi W. Antioxidant activity of crud extracts of Brassica juncea L. seeds on experimental rats. Pharma Innov. 2016;5(8):89–96.
[31] Rahimi HR, Nedaeinia R, Sepehri Shamloo A, Nikdoust S, Kazemi Oskuee R. Novel delivery system for natural products: Nano-curcumin formulations. Avicenna J Phytomed. 2016;6(4):383–98. PMID: 27516979; PMCID: PMC4967834.

[32] Kandeil MA, Mohammed ET, Radi RA, Khalil F, Abdel-Razik AH, Abdel-Daim MM, et al. Nanonaringenin and vitamin E ameliorate some behavioral, biochemical, and brain tissue alterations induced by nicotine in rats. J Toxicol. 2021;2021:4411316. doi: 10.1155/2021/4411316.

[33] Al-Ghamdi NAM, Virk P, Hendi A, Awad M, Elobeid M. Antioxidant potential of bulk and nanoparticles of naringenin against cadmium-induced oxidative stress in Nile tilapia, Oreochromis niloticus. Green Process Synth. 2021;10(1):392–402. doi: 10.1515/gps-2021-0037.

[34] Ahmad A, Prakash P, Khan MS, Altwaijry N, Asghar MN, Raza SS, et al. Enhanced antioxidant effects of naringenin nanoparticles synthesized using the high-energy ball milling method. ACS Omega. 2022;7(38):34476–84. doi: 10.1021/acsomega.2c04148.

[35] Virk P, Alajmi STA, Awad M, Elobeid M, Ortashi KMO, Asiri AM, et al. Attenuating effect of Indian mustard (Brassica juncea) seed and its nano formulation on arsenic induced-oxidative stress and associated genotoxicity in rat. J King Saud Univ Sci. 2022;34(60):102134. doi: 10.1016/j.jksus.2022.102134.