

# Anticancer and Hepatoprotective Role of Selenium Nanoparticles against Liver Carcinogen Acrylamide Induced Toxicity: *In vitro* and *in vivo* Studies

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## ABSTRACT

**Background and Objectives:** Selenium is considered one of the essential components of many enzymes, particularly the antioxidant enzymes such as thioredoxin reductase. Vegetables, among other food sources, are common sources of selenium. In the present study, the green synthesis of Selenium nanoparticles (SeNPs) from *Ocimum basilicum's* aerial part was carried out, as were the anticancer and hepatoprotective roles of selenium nanoparticles against liver carcinogen acrylamide-induced toxicity. **Materials and Methods:** The classification of SeNPs was done by different spectroscopic methods, including UV-spectroscopy, FT-IR, XRD, and HR-TEM. The synthesized SeNPs were tested for their anticancer properties using MTT assays (the normal human liver fibroblast cell line (WISH) and Human liver cancer (HepG2), respectively). **Results:** The histopathological studies indicated that the liver acrylamide induced in mice showed necrosis and damage in liver cells, but the treatment of mice with SeNPs, reduced the effect of acrylamide significantly. **Conclusion:** The results indicated the protective effect of SeNPs on acrylamide-induced cells both *in vitro* and *in vivo*.

**Keywords:** Selenium nanoparticles (SeNPs), Green synthesis, Acrylamide, Cytotoxic activity, Hepatoprotective, Anticancer.

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## INTRODUCTION

The Lamiaceae family includes an annual, herbaceous, 20-60 cm tall, white, and purple flowering plant, *Ocimum basilicum*. According to Chalchat and Özcan, the plant was first found in Iran and India.<sup>1,2</sup> *Ocimum* plant is considered an excellent source of antioxidant potential due to the presence of large quantities of secondary metabolites such as polyphenolic compounds.<sup>3</sup> According to pharmacological surveys,<sup>4</sup> a wide variety of terpenoids and phenolic chemicals were stored in glandular trichomes (specialized glands) present on the surface of their

leaves. The quantity of volatile oils is influenced by plant nitrogen availability, which is a key element in yield.<sup>5</sup> The literature frequently refers to rosmarinic acid as the most prevalent basil phenolic component, while derivatives of chicoric acid and other caffeic acids are also present in significant quantities.<sup>6-8</sup> Since Selenium (Se) is an essential component of the antioxidant enzymes (glutathione peroxidase and thioredoxin reductase), it has recently gained prominence as one of the most essential dietary supplements. Due to their function as antioxidants that scavenge Reactive Oxygen Species (ROS), these enzymes are extremely valuable.<sup>9,10</sup> Oxidative stress and ROS play a substantial part in cancer, and adequate selenium may also play a major role in normal cell growth<sup>11</sup> Inorganic versions of some Se supplements, in particular, have documented toxicity at larger doses. As a result, this trace element's Nanoparticles (NPs) were created to reduce their toxicity and improve their biological activities. In this study, we studied *Ocimum basilicum's* potential for green SeNP



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production. Then, the cytotoxic and hepatoprotective properties of the improved green SeNPs were examined.

## MATERIALS AND METHODS

### Experimental

#### Chemicals

The chemicals used in the study were purchased from S (USA). For qualitative and quantitative evaluations, the following devices and frameworks were used: The WARING Lab Blender (Dynamics Corp., USA), the Hei-VAP Rota vapor (Germany), the LC: 1260 Infinity II (Agilent, USA), the 150/vt Ultrasonic homogenizer (Biologics, North Carolina), the PANalytical X'Pert PRO X-ray Diffraction System (Netherlands), the JEOL JEM-2100 High-Resolution Transmission Electron Microscope (Ja: Shenzhen Mindray Bio-Medical Electronics, China). Sigma-Aldrich

#### Plant Collection

Aerial parts of *O. basilicum* were collected in Tanta, Tanta Governorate, Egypt, and identified in the faculty of science's plant taxonomy department.

#### Plant Extraction

The collected plant samples were thoroughly cleaned with distilled water to remove any pollutants before being dried at 25°C in the dark room. After shade drying, the aerial part of the plant sample was finely ground and used for further studies. The aqueous extract was made using the cold maceration method.<sup>12</sup> Briefly 250 g of the plant powder was thoroughly mixed in a shaker for continuous agitation at 100 rpm after being soaked in 4L of distilled water for 24 hr at 20°C. The extract was then filtered and kept at -4°C for further analysis.

#### HPLC Analysis

The extract of *O. basilicum* was analyzed by HPLC (high-performance liquid chromatography) with a multi-wavelength detector at 280 nm polyphenolic components.<sup>13</sup> A C18 column was used to separate the 10 µL of the sample that was injected at 35°C. Water and acetonitrile were the components of the mobile phase (A and B), (a linear gradient at 1 mL/min: 12-14 min (80% A), 15-16 min. (80% A), 0-5 min. (40% A), 8-12 min. (50% A), and 16-20 min. (80% A) detect and quantify.

#### Green Synthesis of Selenium Nanoparticles

Using a magnetic stirrer, 50 mL of double-distilled water (heated to 60-80°C) was used to dilute the crude aerial portions of *O. basilicum* extract (approximately 30 mL), followed by the addition of 30 mL of a 40 mM selenium acid solution ( $H_2SeO_4$ ), and then at 27°C, the mixed solution was agitated for 24 hr. until the color

changed from pale yellow to crimson. This combination was kept at 27°C in a hot air oven for 24 hr. The obtained SeNP product was centrifuged at 20,000 rpm for 20 min with Double-Distilled Water (DDW) to eliminate contaminants. The resulting crimson pellets were employed for further research after being dried in a freeze-dryer for two days in an airtight container.<sup>14</sup>

#### Characterization of SeNPs

The synthesized SeNPs were principally examined by Rigol Ultra-3660 UV-vis spectroscopy [200-800 nm]. The functional groups and different phytochemicals involved in the reduction process and stability of the produced SeNPs at 4000-400  $cm^{-1}$  were then identified by using FTIR in the Attenuated Total Reflectance (ATR) mode. The size and crystallite of SeNPs were examined by using CuK1-X Ray diffractometer radiation ( $=1.5406 \text{ \AA}$ ) running at 40 kV and 30 mA with 2 varying from 30°-140°, SeNP pellets were coated on a copper grid after being suspended in ethanol, sonicated, and allowed to dry before being investigated with a JEOL-2100 HR-TEM and FE-SEM with EDS mapping analysis (JEOL 7401 F).

#### In vitro anticancer Activity

##### Cell lines

Human liver carcinoma (HepG2) and the liver of a normal human fibroblast cell line (WISH) were acquired from a tissue culture Lab at VACSERA Institute, Agoza, Egypt.

##### Cell culture and MTT assay

To evaluate the cytotoxicity of SeNP, the MTT (3-(4, 5-dimethylthiazol-2-yl)- 2, 5-diphenyl tetrazolium bromide) colorimetric assay was performed in 96-well plates.<sup>15</sup> The entire procedure was kept under sterile conditions by using a laminar air-flow cabinet, following culturing and sub-culturing techniques adopted by Thabrew, *et al.*<sup>16</sup>

#### In vivo anticancer Activity

18 mature male albino rats were used for the *in vivo* studies. Separate cages with a humidity level of between 55 and 65% were used to keep the animals. There were three groups of six animals each. In the animals of groups B and C, hepatocellular carcinoma was induced using Diethyl Nitrosamine (DEN) (50 mg/kg) and acrylamide (2 mg/Kg), which led to oxidative stress as evidenced by an increase in Malondialdehyde (MDA) and a super-decrease in Glutathione (GSH) content. Group (A) served as normal control. Histopathological evidence confirmed the hepatocellular carcinoma's progression. After four weeks, rats were intraperitoneally injected with a single dosage of acrylamide. Group (C) received corresponding treatments with Selenium Nanoparticles (SeNPs).

## Sample collection and/or *in vivo* liver function analysis

The animals were sacrificed by the method of decapitation after eight weeks of treatment. On days 15 and 30 of the treatment period, the cardiac puncture was utilized to collect 5 mL of blood in gel tubes. In a 14-k Humax centrifuge, samples were spun at 3000 rpm for 15 min. Using standard reagents provided by Human Germany, levels of blood glucose were examined on a Humalyzer 3000. Sections of the liver's lobes were preserved in a 10% formalin solution for histological analysis of any cancerous lesions. At the start and end of the experiment, blood (5 mL) was drawn from the vein, and liver function tests, including AST, ALT, and ALP, were performed (Table 1).

## Statistical analysis

SPSS version 16 was used to perform the statistical analysis. All values were expressed as mean±standard error to the mean and were compared with the values of control by one-way ANOVA. *p* values less than 0.05 were considered statistically significant, and values less than 0.01 were considered highly significant.

## RESULTS AND DISCUSSION

### HPLC Analysis

Several polyphenols, including catechin, caffeine, caffeic acid, and quercetin, were found in the samples in the HPLC analysis (Figure 1). Table 2 displays the concentrations and structures of polyphenols. The aerial portions of *O. basilicum* contain significant amounts of chlorogenic acid (962.12 g/g), catechin (904.19), and quercetin (922.23 g/g). There were some unidentified peaks, which could not be identified due to the low concentration. These polyphenolic chemicals are crucial for the synthesis of selenium nanoparticles. Additionally, *O. basilicum*'s 4',7-dihydroxy isoflavone, a polyphenolic molecule, is what gives selenium nanoparticles their ability to last for 18 months without aggregating. Propyl gallate and naringenin were detected in high concentrations in the majority of phenolic compounds. It has been discovered that phenolics are efficient H-donors, (due to the presence of a hydroxyl function group), which accounts for a wide range of biological activities.

**Table 1: Liver function test in presence of selenium and SeNPs in Control group (A), Acrylamide treated group (B) and SeNPs treated group (C).**

Groups	Parameters		
	AST	ALT	ALP
Control group (A)	71.50±1.11	59.67±0.27	292.13±0.12
Untreated group (B)	291.51±1.21	79.21±1.59	421.11±0.31
Treated group (C) Selenium nanoparticles	69.02 ±0.51	57.31±4.11	191.41±1.02

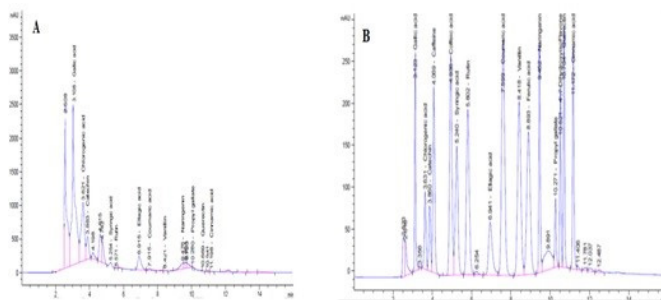
## Green synthesis of SeNPs

The conversion or reduction of selenic acid to Selenium dioxide nanoparticles ( $\text{SeO}_2$ ) was indicated by the change in color of the solution and the formation of a red pellet precipitate.

## U.V. Spectrophotometric Analysis and FTIR Spectroscopic analysis

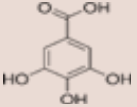
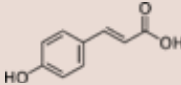
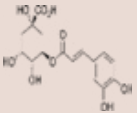
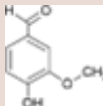
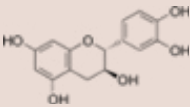
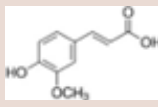
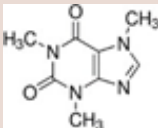
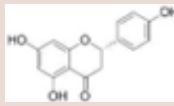
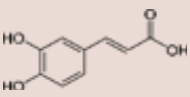
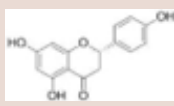
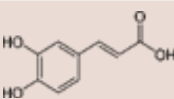
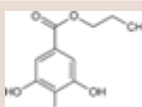
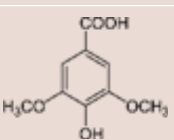
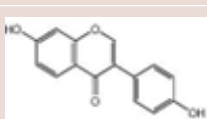
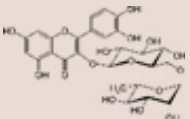
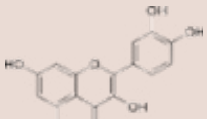
Metal nanoparticles are elucidated due to the differences in solution color and peak locations. UV-vis absorbance spectroscopy has proven to be a very important technique for the investigation of MNPs because of its sensitivity to the size of nanoparticles. The formation of SeNPs was confirmed by the UV-vis spectrum (200-800 nm). U.V. spectrum of SeNPs prepared by green synthesis exhibited a characteristic peak at 282 nm. The calculated direct band gap ( $E_g$ ) was found to be 3.32 eV (Figure 2A).

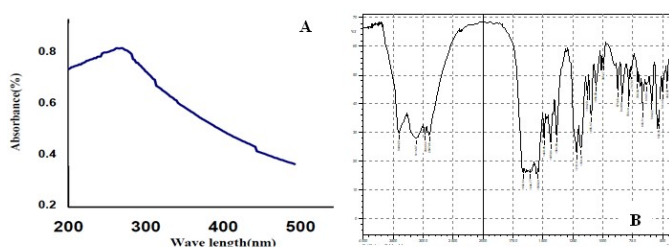
The FTIR technique is usually used to confirm the formation of nanoparticles, and it offers an impression of the vibrational and rotational modes of the existing molecules. FTIR analysis was used to detect the functional groups involved in the reduction and stabilization of SeNPs. Figure 2B depicts the FTIR spectrum of SeNPs, which showed a broad vibration band at  $3406\text{ cm}^{-1}$  corresponding to the O-H stretch of alcoholic and phenolic groups. A characteristic band at  $2330\text{ cm}^{-1}$  corresponds to nitro compounds (N-O asymmetric stretch) present in the compounds, and the absorption band at  $2966$  and  $2897\text{ cm}^{-1}$  represent the C-H stretch of alkynes groups. The strong bands at  $1539$  and  $1489\text{ cm}^{-1}$  are due to the aromatics and alkanes ring (C-C and C-H stretching). Another characteristic band at  $1384$ - $1219\text{ cm}^{-1}$  corresponds to the bending O-H stretches of carboxylic groups,



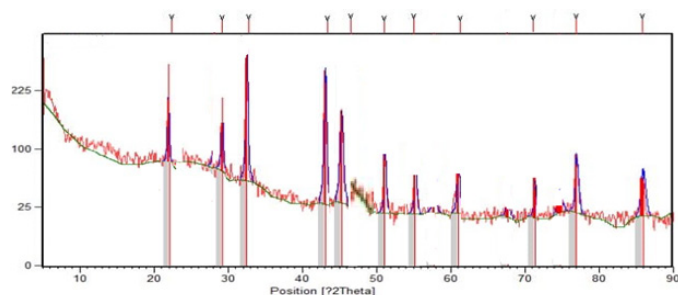
**Figure 1: HPLC chromatogram of, A) polyphenolic compounds of aerial parts of *O. basilicum*; B) standard.**

**Table 2: Concentrations and structures of components of aerial parts of *O. basilicum*.**

Compound	Structure	Conc. ( $\mu\text{g/g}$ )	Compound	Structure	Conc. ( $\mu\text{g/g}$ )
Gallic acid		354.19	Coumaric acid		8.18
Chlorogenic acid		962.12	Vanillin		1.11
Catechin		904.19	Ferulic Acid		0.00
Caffeine		154.24	Naringenin		68.15
Caffeic acid		0.00	Propyl Gallate		7.10
Syringic acid		10.20	4',7-Dihydroxy isoFlavone		932.14
Rutin		20.16	Quercetin		922.23
Ellagic acid		254.14	Cinnamic Acid		19.15

**Figure 2:** (A) UV spectrum of SeNPs, (B) FTIR spectrum of SeNPs.

therefore, these functional groups indicate and confirm the involvement of different reducing and stabilizing agents in the synthesis of SeNPs.<sup>17,18</sup> In the FTIR spectrum, the presence of other bands may indicate the existence of enzymes, proteins, and metabolites such as flavonoids, polyphenols, and carboxylic acid, which remained bound to SeNPs and assisted in the reduction of zinc ions to ZnO.NPs.

**Figure 3:** X-ray diffraction (XRD) Analysis.

### X-ray diffraction (XRD) analysis

The spectrum of the X-ray diffraction pattern of synthesized SeNPs is depicted in Figure 3. The crystalline peaks appeared at  $(2\theta)$  peaks angles of  $23.46^\circ$ ,  $29.18^\circ$ ,  $43.16^\circ$ ,  $46.08^\circ$ ,  $51.61^\circ$ ,  $55.17^\circ$ ,  $61.44^\circ$ ,  $71.11^\circ$ , and  $78.32^\circ$  correspond to the reflection from (100), (101), (110), (102), (111), (201), (003), (202), and (210) crystal



planes, respectively. The crystalline nature of SeNPs is evidenced by this reflection, which is in agreement with the literature.<sup>19</sup>

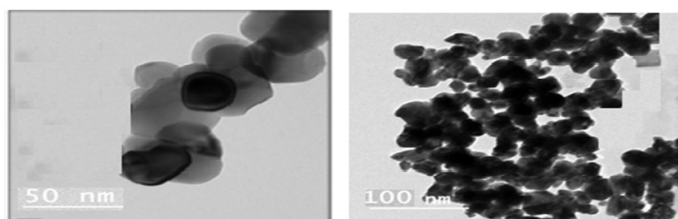
### HR-TEM Analysis

The spectrum of HR-TEM was used to explain the crystalline characteristics and size of the synthesized SeNPs. The HR-TEM analysis was conducted using JEOL-2100, and the images at different magnifications (50 and 100 nm) are shown in Figure 4. TEM images showed individual and spherical nanostructures as the major series of particles that were between 18.96 and 36.28 nm.

### Biological activity

#### *In vitro* assay

A MTT assay was carried out to investigate the cytotoxicity of SeNPs on normal and liver cancer cell lines. Doxorubicin was used as the positive control. The IC<sub>50</sub> inhibitory concentration (50%) was 19.1 µg/mL for HepG2 and 68.3 µg/mL for WISH cell lines. The cytotoxicity results clearly indicated that the SeNPs have selective toxicity towards the liver cancer cell-line (HepG2) in comparison with the normal cell-line WISH. The IC<sub>50</sub> value of doxorubicin was 13.7 against hepG2 cell lines, which is almost similar to the IC<sub>50</sub> of SeNPs, which indicates that SeNPs can be



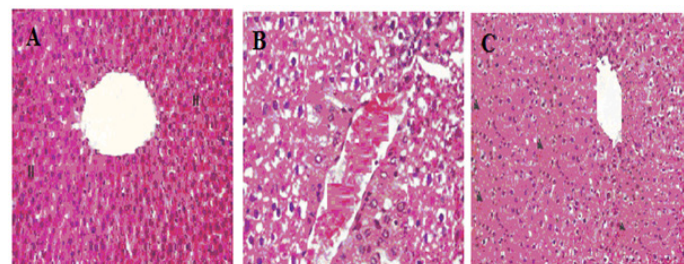
**Figure 4:** HR-TEM analysis of SeNPs at different magnifications.

one of the important anticancer drug candidates against liver cancer (Table 3).

#### *In vivo* assay

#### Biochemical analysis

The selenium nanoparticles' *in vivo* anticancer potential was assessed against human liver cancer cell lines. The findings of this investigation demonstrated that synthetic SeNPs have powerful and selective anti-liver cancer cell line action (HepG2). SeNPs were therefore examined *in vivo* against the same cell line. The studies on liver enzymes were performed, and the levels of liver enzymes were monitored. The levels of AST, ALT, and ALP were 69.02±0.5, 57.31±4.11 and 191.41±1.02 respectively in the SeNP-treated group, 71.50±1.1, 59.67±0.27, 292.13±0.12 in the control group, group 291.51±1.21, 79.21±1.59, and 421.11±0.31 in the positive control. The results significantly indicated that the SeNP-treated group showed all three enzymes in the normal range, which means SeNPs were protecting the liver from the damage caused by Diethyl Nitrosamine (DEN) and acrylamide (Table 1).



**Figure 5:** Effect of SeNPs on liver sections of different groups: A) of control rats revealed normal histological; B) induced control group (acrylamide); C) experimental group treated with SeNPs.

**Table 3:** Cytotoxicity studies (MTT assay) of SeNPs on different cell lines (% viability).

Sample conc. (µg/mL)	Viability %			
	SeNPs		Doxorubicin	
	HepG2	WISH	HepG2	WISH
1000	9.110.27	29.431.89	11.210.59	14.781.68
500	21.250.69	38.221.24	25.171.35	23.680.68
250	36.511.18	41.192.27	40.121.34	25.041.31
125	52.371.45	71.482.16	47.530.09	38.161.08
62.5	64.242.31	88.511.97	69.160.76	49.681.82
31.25	81.010.41	97.621.82	88.632.31	72.651.19
15.6	91.130.65	100	100	88.042.32
7.8	100	100	100	97.110.95
3.9	100	100	100	100
2	100	100	100	100
0	100	100	100	100
IC <sub>50</sub> (µg/mL)	19.1±1.2	68.3±2.9	13.7±1.54	62.1±1.8

## Histopathological examination

Hepatocellular carcinoma-affected livers displayed enlargement, granular, and vacuolar degeneration of the hepatic cells, along with the presence of numerous necrotic cells. The necrotic cells either had pyknotic nuclei or seemed homogeneous in structure without any nuclei. The cells had multiple areas of vacuolar degeneration, and their nuclei were atypical. Rats from Group 1 showed normal hepatic lobule histological structure under a microscope. SeNPs treatment demonstrated moderate granular and vacuolar degeneration, necrosis of hepatic cells with some nuclear pyknosis and activated Kupffer cells, as well as very good repair of the hepatic parenchymal cells, which appeared close to normal with a few scattered necrotic cells. The histopathological studies validated the results of the liver function test and cytotoxicity test. In all the above studies, SeNPs were shown to have significant anticancer as well as hepatoprotective effects both *in vitro* and *in vivo* (Figure 5).

## CONCLUSION

Selenium is one of the most important micronutrients and is an essential part of various enzymes. A deficiency of selenium causes susceptibility to oxidative stress in animals and humans.<sup>20</sup> Selenium nanoparticles showed anticancer activity, which could be due to their presence in antioxidative enzymes. Oxidative stress decreases drastically, which is one of the most common causes of carcinogenesis.<sup>21</sup> The present study clearly indicated that the green synthesized SeNPs exhibit both *in vitro* and *in vivo* anticancer and hepatoprotective effects.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**SeNP:** Selenium nanoparticles; **DEN:** Diethyl Nitrosamine; **MTT:** 3-(4, 5- dimethylthiazol-2-yl)- 2, 5-diphenyl tetrazolium bromide; **ATR:** Attenuated Total Reflectance; **DDW:** Double Distilled Water; **HPLC:** High-performance liquid chromatography; **ROS:** Reactive Oxygen Species; **Se:** Selenium.

## SUMMARY

- Present study was design to synthesize the Selenium Nanoparticles (SeNPs) from *Ocimum basilicum's* aerial part and to perform the anticancer and hepatoprotective activity.
- The purifications and classification of SeNPs was performed by using several advanced techniques including UV-spectroscopy, FT-IR, XRD, and HR-TEM.
- After characterization the SeNPs were studied for their anticancer and hepatoprotective properties.
- The studies indicated that the SeNPs play very crucial role as anticancer and hepatoprotective agents.

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