Original Article

Therapeutic Role of *Sargassum vulgare* with Nano Zinc Oxide against Gamma-radiation-induced Oxidative Stress in Rats

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Abstract

Aim: For more effective therapy and accurate diagnostic instruments and devices, it is crucial to develop novel nanomaterials with excellent biological performance and low toxicity. Due to their outstanding biocompatibility, feasibility, little environmental impact, affordability, and low toxicity, ZnO nanoparticles (NPs) have emerged as one of the most widely used metal oxide NPs in biological applications. One of the many multifunctional inorganic NPs is ZnO, which has been produced using an easy, environmental-friendly process. ZnO NPs have emerged a promising potential in biomedicine, especially in the fields of anticancer and antibacterial fields, which are involved with their potent ability to trigger excess reactive oxygen species production, release zinc ions, and induce cell apoptosis. **Materials and Methods:** Several methods were used to explore the physicochemical properties of ZnO NPs. By using diffuse reflectance spectroscopy, energy-dispersive X-ray, X-ray diffraction analysis, Fourier transform infrared spectroscopies, and scanning electron microscope (SEM), a sample's chemical and elemental composition, crystalline structure, optical properties, and surface appearance have all been studied. Ultraviolet-visible spectroscopy is the main technique for characterizing ZnO NPs. **Results:** A heterogeneous surface form for extremely pure, completely crystalline, and photoactive ZnO NPs was produced. Radiation affects living cells and has an effect on all biological processes in the human body, causing living cells to be damaged. As a result, there is a great deal of interest in developing antioxidant bio-drugs based on *Sargassum vulgare* and ZnO NPs to protect radiotherapy patients and specialists from the dangers of γ-radiation. A major genus of brown marine algae, *S. vulgare,* is found along the Mediterranean and red sea coasts and is a member of the Sargassaceae family. *S. vulgare* methanolic extract (4 g/kg b.wt) and ZnO NPs (10 μM) were given intraperitoneally twice weekly for 6 weeks to rats that had previously received a single dose of γ-radiation (6 Gy) after 1 week of the experiment. There were five groups of rats (15 rats each). **Conclusions:** This cosmopolitan seaweed is known for valuable nutraceutical benefits but has not yet been researched in this regard. As a result, the current study was designed to assess the feasibility and *in vivo* potential activity of *S. vulgare* methanolic extract as a functional food supplement with ZnO NPs in alleviating γ-radiation-associated oxidative damage and toxic symptoms. Based on the findings, *S. vulgare* with ZnO NPs could be used as a therapeutic medication during radiotherapy to reduce the oxidative stress, toxicity, and damage caused by γ-radiation.

Keywords: Gamma-radiation, oxidation stress, rat, *Sargassum vulgare*

Introduction

Zinc oxide, which is used in a growing number of industrial products such as cosmetics, coatings, rubber, and paint, is one of the most important metal oxide nanoparticles (NPs). Due to their superior biocompatibility, affordability, and low toxicity, over the past 20 years, ZnO NPs have developed into one of the most frequently employed metal oxide NPs for biological uses.[1] Zinc is also well known for maintaining insulin's structural integrity. Thus, the development of ZnO NPs for the treatment of diabetes has proven effective. In addition, ZnO NPs have premium luminescent characteristics, making them one of the top contenders for bioimaging.[2] All

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body tissues, including the muscle, bone, skin, and brain, are known to contain considerable levels of zinc, an important trace element. The body uses zinc in its metabolism and it is a key component of several enzyme systems that are essential for the production of nucleic acids, proteins, neurogenesis, and hematopoiesis.[3] Particularly in the areas of antibacterial, anticancer, antidiabetic, antioxidant, wound healing, and anti-inflammatory properties, as well as for use in bioimaging and medication administration, ZnO NPs have shown promise in biomedicine.[1,2,4,5] The risk is caused by the interaction of γ-irradiation with important biological compounds such as DNA, proteins, and lipids via both direct and indirect mechanisms.[6,7] In order to increase oxidative stress, which in turn causes increased organ dysfunction owing to cellular damage, the indirect pathway is crucial.[8,9] Symptoms of acute exposure to large amounts of gamma-radiation are frequently severe.[10] The entire cellular system is stimulated to undergo radiolysis of water by gamma-radiation, which increases the generation of reactive oxygen species (ROS), produces a significant redox imbalanced, and intensifies oxidative stress in all tissues.[11] According to two theories, exposure to gamma-radiation kills live cells directly by ionizing DNA and indirectly by creating excessive amounts of reactive oxygen and nitrogen species that cause cell death.[9,12] Therefore, there is a considerable interest in creating antioxidant bio-drugs employing *Sargassum vulgare* in combination with certain nanometal complexes to protect patients getting radiotherapy and specialized professional workers against the hazard effect of γ-irradiation.[9] An important genus of brown marine algae, *S. vulgare,* is a member of the family Sargassaceae and makes up a significant portion of the Egyptian algal flora growing along the Mediterranean and red sea coasts.[13] Seaweeds are being marketed as functional foods or nutraceuticals since it is generally recognized that they are an incredible source of various health-promoting bioactive chemicals that can act on a wide spectrum of diseases and/or disorders in addition to their fundamental nutritional capabilities.[14] They have been a significant component of the traditional human diets for thousands of years.[13] Meroterpenoids, phlorotannins, fucoidans, sterols, and glycolipids are a few of well-known bioactive substances found in Sargassum. According to reports, it has anti-inflammatory, anticancer, antibacterial, neuroprotective, hypolipidemic, hypoglycemic, anticoagulant, antifungal, antimelanogenic, and hepatoprotective properties.[15-17] There have apparently been numerous pharmacologically and physiologically active compounds extracted, separated, and described from various types of Sargassum, including sulfated polysaccharides, sargaquinoic acids, terpenoids, flavonoids, sterols, polyphenols, pheophytin, and protein. The biological effects of these substances included fibrinolytic activity, inhibition of acetylcholinesterase, antimicrobial activity, induction of hydrozoan larval settlement, analgesic, antioxidant, neuroprotective, antitumor, immune-modulatory, anticoagulant, hepatoprotective, antiviral activity, anti-inflammatory, and cell toxicity.[18-20] Numerous studies have shown that a high intake of natural phenols in the diet and the presence of various antioxidants, such as flavonoids,[21] which are frequently found in seaweeds, reduce the risk of developing some chronic diseases and different types of cancer. This makes seaweeds an excellent source of new drugs with potentially lower toxicity, which has a significant impact on longer life expectancy.[22-24] The Sargassum species, one of the most popular tropical and subtropical brown seaweeds, have a great deal of potential to be used in the industry for nutritional supplements and pharmaceutical fields because they are a very rich source of carotenoids, polyunsaturated fatty acids, iodine, minerals, vitamins (A, B_1, B_2, B_3, B_{12}, C, D, E, and E), proteins, dietary fibers, and essential amino acids.[20] There has been no prior research into the potential protective effects of *S. vulgare* methanolic extract as a food ingredient combined with ZnO NP supplements to reduce oxidative damage and toxic symptoms associated with γ-irradiation. Our findings demonstrated that *S. vulgare* with ZnO NPs can be employed as a natural therapeutic medication during radiation owing to its significant nutrition advantages, safety, and inexpensive.

Materials and Methods

Synthesis of ZnO nanoparticles

In 50 ml of double-distilled water, 1 mM of zinc acetate, 99% pure ($Zn[O_2CCH_3]_2[H_2O]_2$), purchased from Applichem, Germany, was dissolved, and the mixture was then stirred magnetically for 20 min. After that, 25 ml of sodium hydroxide solution was gradually added to the zinc acetate solution. The reaction mixture's color changed after 1 h of incubation. A milky white hue emerged after 2 h of incubation in a magnetic stirrer with the reaction mixture, confirming the biogenesis of ZnO NPs.[25] The precipitate that resulted from this process was removed from the reaction mixture by centrifugation at 6000 rpm and 60°C for 15 min. Next, pellets were collected, dried in an oven with heated air for 2 h at 80°C, and stored at 4°C for further analysis.[26]

Characterization

X-ray diffraction analysis (XRD) (Shimadzu 610, Europa GmbH) was used to characterize the crystal phase information from 20° to 70° using Cu Kα (λ = 0.1546 nm) radiation. For morphological analysis, the scanning electron microscope (SEM) type JEOL 5910, Japan, was used. Using the 20 keV, INCA 200 (UK) energy-dispersive X-ray (EDX) model, the percentage composition and purity were examined. The band gap was determined using a Tauc plot and the 200–1000 nm wavelength range of the Lambda 950 diffuse reflectance spectroscopy (DRS), NICOLET iS10 model with an integrating sphere. The functional group of the compound was examined using Fourier transform infrared spectroscopy (FTIR), Shimadzu, Prestige 21 (Shimadzu Europa GmbH), and spectra in the 400–4000 cm^{-1} range were acquired.[27] The ultraviolet-visible (UV-Vis) absorption spectrum was recorded using a Shimadzu UV-Vis 2600 spectrophotometer. UV-Vis

diffuse reflectance spectra were observed by UV-Vis DRS, Shimadzu 2450, Europa GmbH, in the wavelength range of 200–800 nm.

Algal sampling, identification, and extraction

On October 2021, samples of the brown seaweed *S. vulgare* (Fucales, Phaeophyta) were taken from rocky shorelines in the littoral zone of Hurghada City, Egypt (27° 15' 58.45" N, 33° 48' 57.09" E). The thallic morphology of *S. vulgare* was identified.[28] In the field, Thalli were gathered, thoroughly washed to eliminate any sand particles and epiphytes, stored in clean, sterilized bottles, and then brought cold in the ice box to the laboratory. The algal samples were thoroughly cleaned once again in the laboratory using water that is available at the tap to remove any dirt, sand particles, and salt and then let air dry in the shade at room temperature. Using an electric blender, the samples were ground into a fine powder and then kept cool until further research in sterile, clean plastic bags. Methanol was used as a polar solvent for extracting antioxidant polyphenols.[13] The method described in O'Sullivan *et al.*'s study[29] was used to create the methanolic extract of *S. vulgare* (1:10 w/v). Methanol (99%) was used to suspend the ground seaweed throughout the extraction process, which was then incubated at 180 rpm in an orbital shaker. To achieve thorough extraction, *S. vulgare* was dissolved in methanol (1:10 w/v) and filtered 24 h later after the crude algal methanolic extract had been incubated for 6 h using filter paper Whatman No. 1. Once the solvent extracts have been combined, methanol is eliminated using a rotary evaporator set to 40°C. These crude extracts were additionally lyophilized and freeze-dried. Before being used, crude extracts of *S. vulgare* were kept in liquid nitrogen at −20°C. The antioxidant activity of the crude extracts of *S. vulgare* was identified by applying the subsequent *in vitro* antioxidant assays: $ABTS^{+}$ (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]), DPPH (2,2-diphenyl-1-picrylhydrazyl), and radical-scavenging activities. Furthermore, ferric-reducing antioxidant power (FRAP). As will be discussed later, *S. vulgare* extract was dissolved in distilled water for the *in vivo* study employing experimental rat models.

Test for acute toxicity (LD_{50}) of the *Sargassum vulgare* methanolic extract

Chronic toxicity (LD_{50}) for the *S. vulgare* methanolic extract was calculated using the technique described in Shakuntala *et al.*'s study.[30] *S. vulgare* methanolic extract (100 µl) was administered intraperitoneally to 80 healthy male Wister albino rats (180–200 g) at concentrations of 0.25, 0.50, 1, 2, 3, 4, 6, 8, and 12 g/kg b.wt. For a week, 0.9% NaCl was utilized as a resuscitation agent and/or as a channel for the *S. vulgare* methanolic extract. Rats were closely monitored for any indications of toxicity, and if any appeared, mortality data for 1 week were recorded. The following formula was used to determine the LD_{50} dose regarding the highest (survival) and lowest (death) dose levels:

LD_{50}: $(M_0 + M_1)/2$

whereas M_1 (minimum dose) induced death (mortality) and M_0 (maximum dose) of the survival effects caused by the *S. vulgare* methanolic extract (no mortality).

Metabolomic profiling and antioxidants in a methanolic extract of the seaweed *Sargassum vulgare*

Flavonoids and phenolics were determined by high-performance liquid chromatography (HPLC) (Waters, USA) using the technique described in Abdel-Farid *et al.*'s study.[31] Condensed tannins (proanthocyanidins) were calculated by the method of Morel *et al*.[32] Saponin was identified by the assay of Ebrahimzadeh.[33] Crude protein content was identified by Bradford.[34] ABTS, DPPH, and FRAP contents by the procedure of Sayed *et al*.[9] β-carotene was calculated by the method of Thaipong *et al*.[35]

Gamma-irradiation source

The source of γ-irradiation was a Gamma cell-40 irradiation unit (^{137}Cs), at the National Center for Radiation Science and Technology, Atomic Energy Authority, Egypt. The exposure rate used was 0.84 Gy min^{-1}. Rats received a single dose of 6 Gy γ-irradiation for the whole body.[9]

Potential of experimental design, treatment of animals, and ethics

Wistar albino, adult male rats ($n = 15 \times 5$), 11–13 weeks old and weighing approximately 185–210 g, were transported from the Research Institute of Ophthalmology (animal house) in Egypt. They were kept in stainless steel cages with good ventilation shields, and 1 week prior to the experiment's start, the rats were acclimated to the experiment's standard circumstances (12/12 h light/dark, 65% RH, and 26°C ± 2°C). All of the animals were given a well-balanced diet throughout the entire experiment, and there was unrestricted access to water. This diet included 60% corn maize, 0.30% vitamins, 23% proteins, 0.65 table salt, 20% soybeans, 3.7% fats, 0.82% molasses, 10% growth additives, and 5% wheat bran.[9] Five groups of rats were randomly assigned (15 rats each). Group I: These rats acted as regular controls, and they were given regular food and water every day for 6 weeks in a row.

Group I: These rats acted as regular controls, and they were given regular food and water every day for six consecutive weeks. Group II: Following 1 week of the experiment, rats were given a single dose (6 Gy) for the whole-body gamma-radiation. Group III. Intraperitoneally, rats were treated with seaweed *S. vulgare* methanolic extract at a concentration of 4 g/kg body weight. Group IV: Rats were subjected to a single dose of γ-irradiation (6 Gy) after 1 week of the experiment, followed by intraperitoneal injections with seaweed *S. vulgare* methanolic extract at a concentration of 4 g/kg body weight for six consecutive weeks, twice per week. Group V: Rats subjected to a single dose of γ-irradiation (6 Gy) after 1 week of the experiment, followed by intraperitoneal injections with seaweed *S. vulgare* methanolic extract at a concentration of 4 g/kg body weight with 100 µl ZnO NPs (10 µM) twice/week for six consecutive weeks. We used ZnO

NPs at a concentration of 10 μM according to the study by Fathy *et al*.[36] The animals in this experiment were subjected to the recommendations for proper care of experimental animals, and specific local institutional protocols for the protection of animals under the supervision of authorized investigators were applied. The protocol was revised and approved by the Ethics Research Committee of the National Center for Radiation and Technology at the Egyptian Atomic Energy Authority in Cairo, Egypt (REC-NCRRT-34A/21).[37]

Preparation of tissues and blood

According to the ethical statement,[38] rats were given a light isoflurane anesthesia for a blood sample. Six weeks after the experiment was finished, blood was drawn from an intrinsic jugular vein using special tubes. As a means of separating clear sera for further biochemical investigation, samples of blood were centrifuged at 500–700 rpm (11–16 min), and separated clear sera for additional biochemical examination were maintained at −20°C. Then, all the rats were sacrificed while still unconscious. Remove each rat's liver with care right away for biochemical examination.

Biochemical analysis

Serum examination

Using a semi-automated analyzer (CCL-3001), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) activities, as well as total protein and albumin concentrations, were determined. A rat alpha-fetoprotein (AFP) enzyme-linked immunosorbent assay kit was used to determine the amount of AFP.[39]

Investigations of liver tissue

It was determined that nitric oxide existed.[40] Malondialdehyde (MDA) levels were assessed using a method described in Yoshioka *et al*.'s study.[41] The method of Ewing and Janero[42] was used to calculate superoxide dismutase (SOD). According to Giustarini *et al*.'s study,[43] reduced glutathione (GSH) levels were evaluated. Catalase activity (CAT) was measured using the procedure described in Aebi study.[44]

Statistical analysis

Statistical analysis was performed with SPSS version 17 statistic software package (SPSS Inc., Chicago, USA). Data were expressed as means ± standard deviation. Significance was determined to be statistically different when $P \leq 0.05$.[45]

Results

Physicochemical study

X-ray diffraction analysis

Bands of diffraction at the 2θ location of ZnO NP XRD spectrum [Figure 1] were matched to reference card 01-079-0205 by the equivalent hkl values of 31.87 (100), 34.14 (002), 36.62 (101), 47.48 (102), 56.79 (110), 63.21 (103), and 67.89 (112). The biosynthesized ZnO NPs were predicted to have an average crystalline size of 37 nm.[46] These reflections were thought to be the cause of the hexagonal form

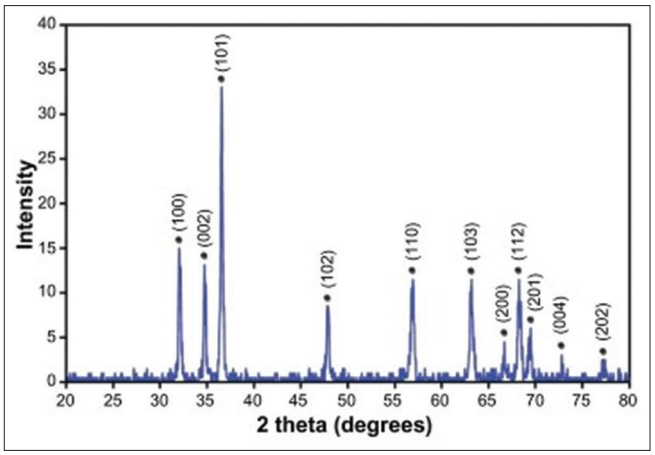

Figure 1: X-ray diffraction analysis spectrum of ZnO nanoparticles

of ZnO NPs. All of the peaks were assigned to the necessary elements, indicating that the synthesized samples are unusually pure, and the sharp and strong diffraction bands show the synthesis of well-crystalline nanostructures.

Ultraviolet-visible spectroscopy analysis

The main technique for characterizing ZnO NPs is UV-Vis spectroscopy. A metal NP's surface plasmon resonance, or collective free electron oscillation within the metal NPs, determines the majority of its optical qualities.[25] The UV-Vis spectra of freshly produced ZnO NPs are presented in Figure 2. UV-Vis of ZnO NP absorption spectra were measured in the range of 300–600 nm. The production of ZnO NPs is clearly indicated by the absorption peak at 358 nm. This outcome matches other studies that have been published.[47,48]

Screening electron microscope of ZnO nanoparticles

The morphology of biosynthesized ZnO NPs was observed by SEM, as shown in Figure 3. The diameter of cluster ZnO NPs was 2 μm and in hexagonal nanochip shape with rough surface.[49]

Energy-dispersive X-ray and Fourier transform infrared analysis

The spectrum of ZnO NPs by EDX [Figure 4] shows three peaks at 0.9, 8.8, and 9.7 keV, indicating that Zn is present, but at 0.25 keV, the peak attributed to O and the absence of any additional peaks suggest that the sample of ZnO is exceptionally clean. The EDX spectrum shows the high values of zinc (80.3%) and oxygen (19.7%), respectively. The FTIR spectrum of ZnO NPs [Figure 5] shows the wavenumbers at 3375, 2349, 1375, 915, and 481 cm^{-1}, respectively. The broad peak at 3375 cm^{-1} indicated the -OH stretching vibrations. The peak at 2349 cm^{-1} indicated the free –C = O group. Stretching vibrations present at 1375 cm^{-1} showed C-O-H bending mode. The peak at 915 cm^{-1} showed the characteristic -NH of amine. The band at 481 cm^{-1} confirmed the stretching vibrations of ZnO NPs.[50,51]

Diffuse reflectance spectroscopy

The transmittance spectra were used to assess the electronic state of the ZnO NPs and revealed that all of the samples are transparent

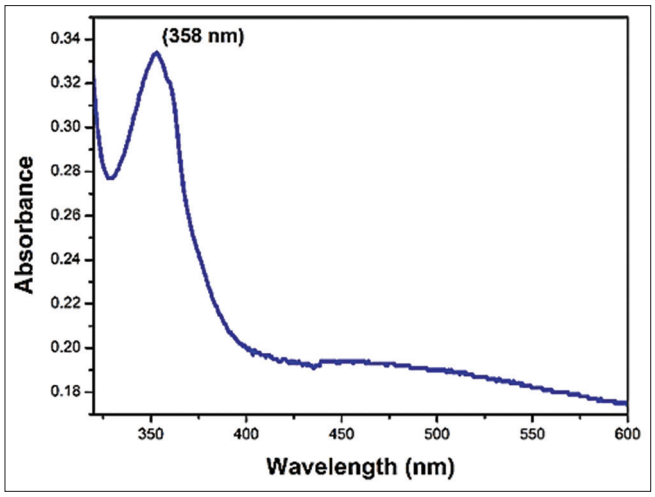

Figure 2: Spectrum of created ZnO nanoparticles in the ultraviolet-visible

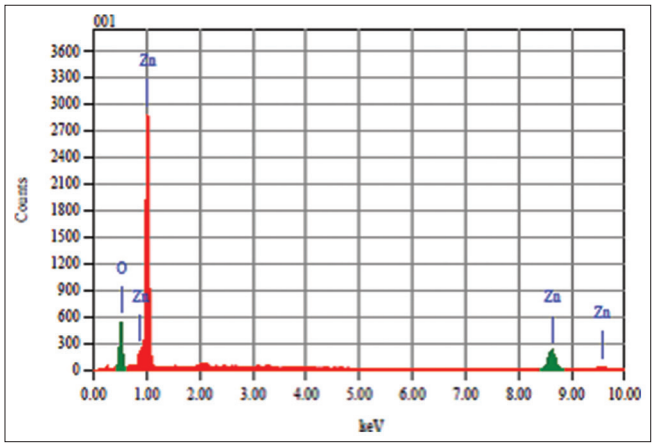

Figure 4: Energy-dispersive X-ray spectrum of ZnO nanoparticles

across a wide wavelength range. The optical band gap energies were calculated using the Tauc relation ($\alpha h\upsilon = B[h\upsilon - Eg]^{n}$), where hv is the light intensity, is the absorption coefficient, B is constant, and *n* varies depending on the type of transition [for bidden direct, direct, forbidden indirect, or indirect, with values of 2, 1/2, 3, or 3/2, respectively, as shown in Figure 6a.[49,51]] DRS spectra of the synthesized ZnO NPs [Figure 6b] show decreased transmission in the UV range (maximum absorption). The band gap energy for ZnO NPs was found from the Tauc plot to range from 3.10 eV to 3.37 eV, which corresponds to emission in the UV region.[52,53]

Test for acute toxicity (LD_{50}) of the Sargassum vulgare methanolic extract

The calculated LD_{50} for the methanolic extract of the seaweed *S. vulgare* was 8 g/kg b.wt. Therefore, it was decided that the ideal dose (precautionary dose) for use throughout the experiment would be half of this quantity (4 g/kg b.wt.), as shown in Table 1. It was determined how this dose affected the various molecular and biochemical variables being studied.

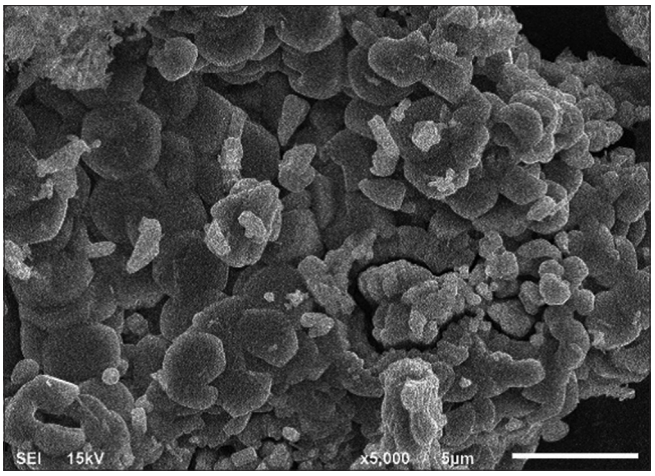

Figure 3: The SEM image of ZnO nanoparticles

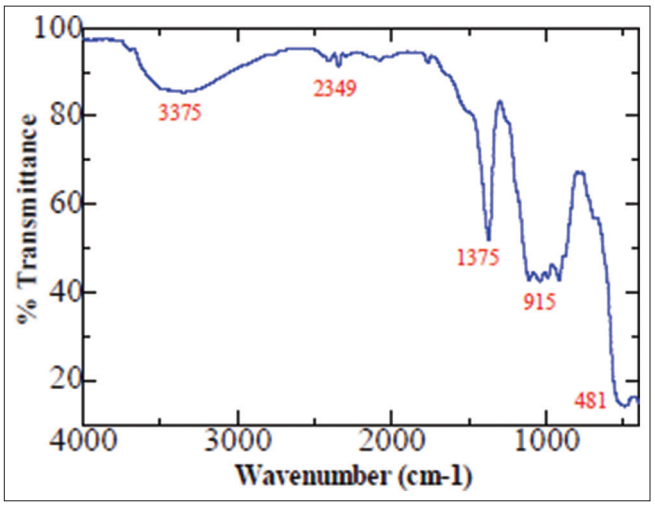

Figure 5: Fourier transform infrared spectroscopy spectrum of ZnO nanoparticles

Phytochemical and metabolomic profiling of the methanolic extract of Sargassum vulgare

Phytochemical and metabolomic profiling of the *S. vulgare* methanolic extract was evaluated for biological activity. The identification of 15 polyphenol compounds (fractions of phenolic and flavonoid compounds) and their concentrations by using HPLC are shown in Table 2. Methanolic extract was chosen for the toxicity study because it has more bioactive phytoconstituents than other solvent extracts, and there is interest in conducting more pharmacological research with *S. vulgare*.[13]

Antioxidant capacity

To ascertain the antioxidant qualities of *S. vulgare* methanolic extract, a variety of assays were used [Table 3]. The antioxidant activity is based on the redox properties of the extracts, which facilitate their activity as reducing agents; such ability is generally associated with the presence of reductants, which exert antioxidant action by breaking the free radical chain by donating a hydrogen atom or preventing peroxide formation.[54]

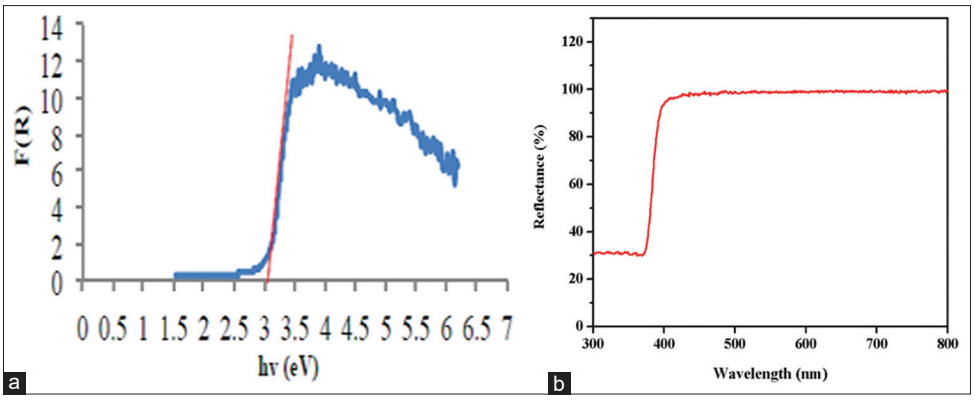

Figure 6: Plot of indirect band gap energy of ZnO nanoparticles (a), diffuse reflectance spectroscopy spectrum of the synthesized ZnO nanoparticles (b)

Table 1: Assessment of lethal dose 50 of the methanolic extract of *Sorghum vulgare*

Groups	Dose (g/kg body weight)	Number of rats	Dead rats	Live rats	Dead (%)	Live (%)
1	0.25	8	0	8	0	100
2	0.50	8	0	8	0	100
3	1	8	0	8	0	100
4	2	8	0	8	0	100
5	3	8	0	8	0	100
6	4	8	0	8	0	100
7	6	8	2	6	25	75
8	8	8	4	4	50	50
9	10	8	6	2	75	25
10	12	8	6	2	75	25

Values are mean of samples in triplicate

Table 2: Estimation of flavonoid and phenolic fractions (μg/g DW) in the methanolic extract of *Sorghum vulgare*

Compounds	RT (min)	Concentration (μg/g DW)
Phenolics		
Benzoic acid	1.1	10±0.43
Gallic acid	1.9	73±1.95
Resorcinol	2.1	14±0.42
Chlorogenic acid	3.0	38±2.18
Caffeic acid	3.6	10±0.52
P-coumaric acid	4.5	36±1.32
Salicylic acid	4.8	63±2.52
Ferulic acid	5.5	53±2.12
Cinnamic acid	6.1	23±0.91
Syringic acid	8.2	24±0.87
Flavonoids		
Catechin	1.1	13±0.53
Kaempferol	2.3	20±0.84
Rutin	3.2	19±0.95
Hesperidin	4.9	27±1.56
Quercetin	6.9	10±0.52

Values are mean±SD of samples in triplicate. RT: Retention time. SD: Standard deviation

The DPPH and ABTS radicals may be noticeably quenched by the methanolic extract of *S. vulgare*, which can also operate as a potent reductant. These antioxidant properties are probably due to the presence of phenolics and flavonoids, which are strong chemicals that function as hydrogen donors and/or electron donors and can interact with ABTS and DPPH free radicals to transform them into byproducts that are more stable. Both phenolics and flavonoids are prevalent in many seaweeds and possess an extensive array of biological and free radical-scavenging abilities, which is consistent with our findings.[55] The ability of various seaweed species to scavenge DPPH radicals varies significantly.[56]

Table 4 shows that the methanolic extract of *S. vulgare* contains a variety of beneficial natural substances such as protein, β-carotene, saponins, and proanthocyanidins (condensed tannins).

Treatment with γ-radiation at 6 Gy significantly increased ($P \leq 0.05$) enzyme activities of liver (ALP, GGT, ALT, AFP, and AST), NO, and MDA. Conversely, as compared to the control γ-radiation application decreased significantly ($P \leq 0.05$) albumin, total protein, antioxidant enzyme activities (SOD, GSH, and CAT). In contrast, *S. vulgare* methanolic extract (4 g/kg b.wt.) application only or with ZnO NPs at a concentration of 10 μM is accurately reflected in these measurements [Tables 5 and 6].

Discussion

An earlier investigation reported that γ-irradiation increased levels of ALT, AFP, AST, GGT, and ALP in the liver.[9] By enhancing the liver cell membrane's oxidation, which results in membrane breakdown and increased permeability, gamma-radiation increased the activities of transaminases. Eventually, transaminases leak into the blood as a result of this process.[57] In contrast, *S. vulgare* methanolic extract (4 g/kg b.wt) application alone or with ZnO NPs significantly decreased liver functions (AFP, GGT, ALT, ALP, and AST), and this is attributed to the powerful antioxidant polyphenolic substances that enhanced synergistic activity, as well as to various extra secondary metabolites.[58] Due to an unbalance between oxidants and antioxidants, the radiolysis caused by γ-radiation produces a large amount of ROS (oxidative stress), in tissues

and the biological system's inability to remove these reactive substances, which eventually cause significant damage to living tissues and lead to organ malfunction.[9,59] Many antioxidants, including polyphenols, saponins, and vitamin E, have been studied in recent years for their potential or actual benefits against oxidative stress.[60] Recently, there has been increased use of natural products of Sargassum vulgare as antioxidants from plant origins due to their safe nature, low cost, and ease of use, as well as to avoid the use of industrial products (excessive costs), which have many harmful effects on human health and cause many diseases.[12,31,58] The current study's findings demonstrated that γ-radiation treatment led to a significantly higher level of MDA and NO, along with a significantly lower level of CAT, GSH, and SOD.[9,12,58] Antioxidant molecule levels were significantly reduced as a result of the radiolysis of water brought on by gamma-radiation.[61] The presence of proanthocyanidin (condensed tannins) in the *S. vulgare* extract dramatically increased SOD activity.[62] Conversely, application of *S. vulgare* extract with ZnO NPs efficiently reduced MDA and NO levels in γ-radiation-treated rats by a significant amount. In addition, they markedly inhibited the activity of the antioxidant enzymes POD, CAT, GSH, and SOD as *S. vulgare* have a highly abundant source of nutritious and bioactive substances, such as polyunsaturated fatty acids, minerals, carotenoids, iodine, vitamins (A, B_1, B_2, B_3, B_{12}, C, D, and E), dietary fibers, proteins, and essential amino acids.[20,58] The polyphenols in *S. vulgare* reduce the amount of nitric oxide.[63] However, application of *S. vulgare* extract with ZnO NPs, effective antioxidants (polyphenols), which prevent free radical generation, as well as the enhancement of the endogenous CAT and SOD genes through influencing signaling pathways that lead to the expression of these crucial enzymes involved in the antioxidant defense system, alleviated this toxic effect.[64] *S. vulgare* is highly recommended as a food ingredient and a source of a variety of useful components, including proteins, sugars, polyphenols, and isoflavones.[65] By inhibiting the generation of free radicals, these substances are essential for protecting cells against ROS harmful effects. Phytochemicals served as a reducing, capping, and stabilizing agent, while

Table 3: Antioxidant capacity of *Sargassum vulgare* (2,2-diphenyl-1-picrylhydrazyl, [+2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)]) radical-scavenging activity and ferric-reducing antioxidant power of seaweed *Sargassum vulgare* methanolic extract

Parameters	Concentration
ABTS (mg VCE/g DW)	185±14.6
DPPH (mg VCE/g DW)	123±8.9
FRAP (mM Fe^{2+} equivalent/g DW)	1.5±0.04

VCE mg. Values are mean±SD of samples in triplicate.
DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), FRAP: Ferric-reducing antioxidant power, VCE: Vitamin C equivalent, SD: Standard deviation

Table 4: Quantitative analysis for the phytochemical constituents of seaweed *Sargassum vulgare* methanolic extract

Parameters	Concentration (mg/g DW)
Condensed tannins	2.27±0.14
Saponins	93.48±4.6
β-carotene	3.7±0.29
Protein	98.7±8.8

Values are mean±SD of samples in triplicate. SD: Standard deviation

Table 5: Changes in liver function (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, alpha-fetoprotein, total protein, and albumin) parameters among the treated animal groups

Groups	ALT (U/L)	AST (U/L)	GGT (U/L)	ALP (U/L)	AFP (ng/mL)	Total protein (g/dL)	Albumin (g/dL)
Group I	59±2.5^{d}	48±1.5^{d}	35±2.8^{d}	99±8.9^{d}	4.5±0.3^{d}	6.5±0.3^{a}	4.6±0.3^{a}
Group II	212±8.8^{a}	197±17.7^{a}	143±7.2^{a}	386±22.5^{a}	18.8±1.3^{a}	3.9±0.3^{c}	2.3±0.1^{c}
Group III	50±2.0^{d}	42±3.4^{d}	31±2.8^{d}	85±5.1^{e}	3.9±0.2^{e}	6.6±0.4^{a}	4.5±0.2^{a}
Group IV	99±5.8^{c}	83±4.8^{c}	59±3.3^{c}	160±6.4^{c}	7.6±0.4^{c}	4.6±0.9^{b}	3.7±0.4^{b}
Group V	113±4.5^{b}	98±4.9^{b}	71±5.7^{b}	200±8.0^{b}	9.2±0.3^{b}	6.2±0.8^{a}	4.1±0.4^{a}

Values are mean±SD of samples in triplicate. Values in the same row followed by the same letter are not significantly different ($P \leq 0.05$). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, AFP: Alpha-fetoprotein, SD: Standard deviation

Table 6: Changes in antioxidant enzymes (superoxide dismutase, glutathione, and catalase activity), malondialdehyde, and nitric oxide among the treated animal groups

Groups	SOD (U/g tissue)	GSH (U/g tissue)	CAT (U/g tissue)	MDA (nM/g tissue)	NO (μM/mL)
Group I	123±7.4^{a}	196±9.8^{a}	94±5.9^{a}	15±0.7^{c}	7±0.2^{c}
Group II	51±3.1^{e}	81±4.9^{e}	42±3.4^{c}	48±2.9^{a}	32±1.9^{a}
Group III	114±10.3^{b}	181±18.2^{b}	89±4.4^{a}	11±0.5^{c}	5±0.2^{c}
Group IV	87±5.3^{c}	141±15.5^{c}	64±3.2^{b}	28±2.3^{b}	15±1.2^{b}
Group V	75±3.8^{d}	121±13.6^{d}	62±3.6^{b}	32±1.7^{b}	14±0.8^{b}

Values are mean±SD of samples in triplicate. Values in the same row followed by the same letter are not significantly different ($P \leq 0.05$). SD: Standard deviation, SOD: Superoxide dismutase, GSH: Glutathione, CAT: Catalase activity, MDA: Malondialdehyde, NO: Nitric oxide

plant polyphenols had a stronger antioxidant capacity.[66] In addition, ZnO is rated as "GRAS" (by the US Food and Drug Administration), a substance that is "generally recognized as safe."[67] MDA and nitric oxide levels may be dramatically reduced by ZnO NPs.[2] It is well recognized that all tissues in the body, such as skin, muscle, bone, and brain, contain significant amounts of Zn (fundamental trace element). Zinc participates in the body's metabolism and is a key component of several enzyme systems, which are essential for the production of neurogenesis, nucleic acids, proteins, and hematopoiesis.[3] For more effective therapy and accurate diagnostic instruments and devices, it is crucial to develop novel nanomaterials with excellent biological performance and low toxicity. The challenge for scientists to reconcile the risky side effects of metal oxide NPs with their advantageous therapeutic effects has grown in recent years.[1-5] Eco-friendliness and biodegradability are ZnO NPs' most notable characteristics.[68] In addition, plant components, including proteins, enzymes, and carbohydrates, are employed to create NPs that interact with biomolecules with ease. Secondary plant metabolites with cytotoxic activity, such as lignin, hemicellulose, pectin, flavonoids, and antioxidant polyphenolic chemicals, are found in plants and can help in the development of new pharmaceuticals.[69] Because it contains numerous essential nutrients, including polyphenolic compounds (radical scavengers), amino acids, B-complex vitamins, carbohydrates, saponins, protein, fatty acids, *β*-carotene, fibers, essential minerals (K, Na, Ca, and Mg), and glucose, *S. vulgare* extract was used and regarded as a significant source of good nutrition.[20,58] Overall, it was shown that the *S. vulgare* extract studied was significantly able to quench the ABTS and DPPH radicals and function as a potent reductant.[55,58] Its phenolic and flavonoid concentrations are probably responsible for these antioxidant capabilities, which have several biological applications and are scavengers of free radicals.[55] Our findings demonstrated the preventive potential of *S. vulgare* methanolic extract containing ZnO NPs on rats that could tolerate γ-irradiation from various angles. The identification of 15 polyphenol compounds (fractions of phenolic and flavonoid compounds) in *S. vulgare* methanolic extract, which are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. In the last decade, there has been much interest in the potential health benefits of dietary plant polyphenols as antioxidants. Epidemiological studies and associated meta-analyses strongly suggest that long-term consumption of diets rich in plant polyphenols offers protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases.[70] Antioxidant activity was evaluated using several *in vitro* assays: total antioxidant capacity, FRAP, and diphenylpicrylhydrazyl radical-scavenging activity. The crude extracts of the algae *S. vulgare* contained phenolic compounds in various proportions and showed certain levels of *in vitro* antioxidant activity and *in vivo* anti-inflammatory activity without toxicity.[71-75] In biological systems, antioxidants are chemicals that interact with and neutralize free radicals, either directly scavenging ROS or serving as electron donors for antioxidant enzymes, thus preventing them from causing damage. Antioxidants are also known as "free radical scavengers." The body makes some of the antioxidants that it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. The antioxidant systems, including enzymatic antioxidants such as SOD, GSH peroxidases, thioredoxin, as well as the exogenous antioxidants, can manipulate the ROS level by regulating the genes expression and related signaling pathways to maintain the redox balance and cellular component integrity.[75]

Conclusions

Our findings demonstrated that *S. vulgare* methanolic extract can be used as a functional food with ZnO NP supplements (a novel food) for protecting patients and occupational workers during radiotherapy as a natural therapeutic medicine because of its beneficial nutritional properties, safety, and affordability. The necessary nanostructures (ZnO NPs) are extremely pure and impurity free, according to the EDX analysis. The XRD result showed the sample was well crystallized in a hexagonal quartzite structure with a crystallite size of 11.9 nm. SEM-EDS analysis showed the morphology of a hexagonal nanochip with the compositions of Zn and O elements. Natural synthesis is less time-consuming and less expensive and requires less expensive equipment than synthetic synthesis. This study looked into the possibility of the edible seaweed *S. vulgare* as a functional food with ZnO NP addition. It has strong antioxidant properties and may be a promising functional food ingredient for treating oxidative damage and harmful effects from γ-irradiation. The powerful antioxidant phenolics (gallic, *p*-coumaric acids, salicylic, chlorogenic, and ferulic), flavonoids (quercetin, rutin, hesperidin, and kaempferol), and plus other polar substances of the macroalga *S. vulgare* with ZnO NPs appear to work in concert to protect against the oxidative stress caused by γ-radiation. These organic antioxidants have the unique ability to scavenge ROS, thereby enhancing the cellular oxidant/antioxidant equilibrium, altering immune defense mechanisms, and ultimately controlling the signaling pathways that initiate pro-inflammatory cytokines and apoptotic proteins.

Statement of ethics

The guidelines for the ethical use and maintenance of laboratory animals issued by the National Institutes of Health and authorized by the Scientific Committee of the Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, were complied with in all procedures used in caring for rats and taking blood and tissue samples for this experiment**.**

Availability of data and materials

All the data generated or analyzed during this study are included in this published article.

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Conflicts of interest

There are no conflicts of interest.

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