Variations of glaucine, quercetin and kaempferol contents in *Nigella arvensis* against Al$_2$O$_3$, NiO, and TiO$_2$ nanoparticles

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**ABSTRACT**

The present study was designed to determine the glaucine, quercetin and kaempferol contents in the root and shoot parts of *Nigella arvensis*, treated for 21 days with different concentrations of the nanoparticles (NPs), including titanium dioxide (TiO$_2$), alumina (Al$_2$O$_3$) and nickel oxide (NiO) by high-performance liquid chromatography (HPLC). Results showed a significant increase in the total flavonoid and total alkaloid content in treated plants. Glaucine content in shoot parts was significantly higher than the root parts. The highest amount of glaucine was obtained in shoots and roots exposed to NiONPs at concentrations of 1000 and 2500 mg/L with up to 3.2 and 2.6 fold increase compared to the control group, respectively. The maximum content of quercetin was observed in the shoot and root parts under 50 mg/L NiONPs with 2.2 and 1.8 fold increase compared to the control group, respectively. The kaempferol content was significantly decreased in all treatment, except for 1000 mg/L NiONPs treatment in the root parts, which was 2.9 fold higher than the control group. Apart from the toxic effects of some NPs, our findings suggest that the NPs at specific levels can be considered as new and appropriate elicitors for *in vitro* production and increasing the biosynthesis of secondary metabolites to use in pharmaceutical applications.

1. Introduction

Metal and metal oxide nanoparticles have received specific attention due to their unique properties and widespread applications in medical, agricultural, industrial, consumer products, and military fields [1]. Nevertheless, the unknown release of these nanoparticles into the ecosystem has caused global concern about their safety, environmental health and potential phytotoxic effects [2, 3]. The physicochemical properties of engineered nanoparticles (ENPs) including size, composition, surface charge, dissolution, and nature of the environmental targeted matrices determine the fate of these particles in the environment [4, 5].

Considering the importance of the interaction of ENPs with the biological systems, nowadays many researchers have investigated the effects of different types of nanoparticles in humans, animals, and plants [3, 6]. The plants are an initial and very important element of ecological systems and play a very vital role as significant ecological receptors in transportation, translocation and accumulation of ENPs into the food chain [7]. A good and precise understanding of the interactions of plant systems with ENPs is very important for evaluating the toxicity and transporting of ENPs in trophic chains [8].

However, the main functional mechanism of ENPs in biological systems is unknown, although the oxidative stress generated by reactive oxygen species (ROS) is proposed as their toxic effects [3]. The production of different antioxidant enzymes such as catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) and non-enzymatic systems with the production of defensive chemical compounds (metabolites) are mechanisms through which plants can protect themselves from oxidative stress, physiological damages, pathogenic attacks and UV radiation [9, 10].

Two types of metabolites are produced by plant cells, including primary and secondary metabolites. Primary metabolites (lipids, proteins and carbohydrates), are directly involved in growth and metabolism and secondary metabolites (alkaloids, flavonoids, phenolics, terpenoids, essential oils, quinones, resins, tannins, lignins, steroids, etc.), are the final products of primary metabolism and play crucial roles in the
protection of plants against diseases, pests and different environmental stresses [11, 12]. Typically, secondary metabolites are synthesized by plants against different biotic or abiotic elicitors/stresses and/or stimulator molecules, which will possibly induce biochemical and physiological processes of the objective plants and activate the defense mechanisms [13, 14].

Thus, ENPs can act as chemical elicitors of plant defensive systems, which are often operated by increased production of secondary metabolites [11]. Many researchers have studied the role of NPs as elicitors [15, 16, 17, 18, 19, 20]. Krishnaraj et al. (2012) indicated that the total phenol content enhanced in Bacopa monnieri (L.) treated with biologically synthesized silver nanoparticles (AgNPs), which it may be one of the defense mechanisms of the plant against the mild stress condition [17]. Also, Khan et al. (2016) investigated the effect of different metal NPs, including monometallic (Ag, Au, Cu) and bimetallic alloy (AgAu, AuCu, AgCu) on contents of total flavonoid and phenolic in the milk thistle plant. They showed that after 28 days of treatment, the maximum contents of total phenolic and flavonoid were obtained against the monometallic NPs as compared to bimetallic alloy NPs [21]. The production of aloin was increased in cell suspension culture of Aloe vera treated with TiO2 and Ag NPs as abiotic elicitors after 48 h elicitation, but its amount was gradually decreased with time [18].

Nigella arvensis L. is an annual herb commonly known as wild fennel or black bread weed and belongs to the genus Nigella and family Ranunculaceae [22]. It is distributed in the Near East region, central and south-east Europe to the Russian Federation and northern Africa [23]. It has medicinal properties such as anti-inflammatory, antiallergic, antiviral and antihelminthic [24], as well as is locally used as a flavor for cakes and bread [25].

N. arvensis contains several active compounds such as alkaloids and flavonoids with various biological activities. For example alkaloids of plant origin were shown to have potent antiviral activity against several viruses, including the hepatitis B virus [26]. The existence of 12 flavonoids has reported in this plant, which most important of them included quercetin and kaempferol [24]. Quercetin belongs to a subclass of flavonoids called flavonols and has pharmacological properties and benefits, including antioxidant, antiadiabetic, antioxidant, antiviral, antibacterial, anticarcinogenic and anti-inflammatory [27, 28]. In addition to the above-mentioned properties, kaempferol has also been applied for the treatment of numerous diseases, such as diabetes, inflammation, anxiety, osteoporosis, neurodegenerative diseases, allergies and infectious diseases as well as cardiovascular diseases and some kinds of cancer [29]. Eight known aporphine alkaloids have been isolated from N. arvensis, which main alkaloids are glaucine and braceline [30]. Aporphine alkaloids belong to alkaloids of the isoquinoline type that process pharmacological activity, including antihypertensive, antidiabetic, antioxidant, antiobesity, and antivirus (anti-HIV's), cytotoxic, immunoregulatory activities and antiplatelet effects [31, 32]. Glaucine is also used in medicine as an antitussive agent [33].

Therefore, the main aim of this research was to evaluate the influence of TiO2, Al2O3 and NiO NPs on biosynthesis and production of secondary metabolites, including the contents of total flavonoids, total alkaloids, glaucine, quercetin and kaempferol in endemic Nigella arvensis by HPLC method.

2. Experimental

2.1. Nanomaterials, seeds and chemicals

Nanoparticles: TiO2NPs (80 vol% anatase +20 vol% rutile), Al2O3NPs (gamma), and NiONPs were purchased from Iranian Nanomaterial Company (Mashhad, Iran). The physical characteristics of Al2O3, NiO and TiO2 NPs reported by the supplier were as follows: NiONPs with the size of 5–8 nm, nearly spherical morphology, purity of 99.5% and surface area 50–100 m2/g, Al2O3NPs (gamma) with the size of 5 nm, nearly spherical morphology, purity of 99.99% and surface area 150 m2/g and TiO2NPs (80 vol% anatase +20 vol% rutile) with a size of 20 nm, nearly spherical morphology, purity of 99% and surface area 10–45 m2/g. Seeds of N. arvensis were purchased from Pakan Bazr Company (Isfahan, Iran). Glaucine (Santacruz Co), methanol (HPLC grade, Merck) and HCl (Sigma–Aldrich Chemical Co) were also supplied. All used organic solvents were of HPLC grade.

2.2. Plant culture and preparation of extract

Seeds of N. arvensis were sterilized before germination (in 10% sodium hypochlorite solution for 10 min). They were germinated on sand soaked with 0.1 strength modified Hoagland's solution. After ten days, the solution was replaced with Hoagland solutions containing different concentrations of TiO2NPs, NiONPs and Al2O3NPs (0, 50, 100, 1000 and 2500 mg/L). Before the replacement, the NP suspensions (100 mL) were first sonicated in an ultrasonic water-bath for 90 min. All experiments were performed in a greenhouse under semi-controlled conditions. All treatments had three replicates, and the experiment lasted for three weeks (21 days). Afterward, the plants were harvested and washed with tap and distilled water. The shoot and root biomass were oven-dried at 65 °C for three days. Extraction was performed by the described method of Zuo et al. [34], with minor modifications. The shoot and root samples of N. arvensis (100 mg) were ground and extracted with 20 mL methanol (80%) containing 0.15% HCl for 3 h with 10 min sonication. The obtained extracts were centrifuged and solutions were separated. Aliquot of 20 μL from the extract solutions were injected into the HPLC system.

2.3. Total alkaloid content

The total alkaloid contents of N. arvensis samples were analyzed by the described method of Singh et al. with slight modifications [35]. Each sample (100 mg) was extracted with 80% ethanol (10 mL) and then was filtered and centrifuged at 5000 rpm for 10 min. Then, 1mL of each extract was mixed with 1mL of FeCl3 solution (0.025M in 0.5M HCl) and 1mL of 1,10- phenanthroline (0.05M in ethanol). The reaction mixture was incubated in a hot-water bath for 30 min at a temperature of 70 ± 2 °C. After cooling down, the absorbance of the red color complex was recorded at 510 nm. Finally, the total alkaloid content was calculated using a standard curve plotted with glaucine, and the values are expressed as mg glaucine (GC)/g of the dry weight of the plant.

2.4. Total flavonoid content

Determination of the total flavonoid content of N. arvensis was performed using the colorimetric method as defined by Quettier–Deleu (2000) with slight modifications [36]. A 1mL aliquot of methanolic extract of each sample was mixed with 1 mL of aluminum chloride solution (2%). After 60 min incubation at room temperature, the absorbance of the reaction mixture was recorded at 415 nm using a spectrophotometer (Thermo Fisher Scientific, model 4001/4). The calibration curve was plotted using quercetin, and the total flavonoid content was expressed as mg quercetin/g of the dry weight of the plant.

2.5. HPLC analysis

All samples were analyzed by an isocratic method using a reversed-phase HPLC system. The used instrument was a Knauer HPLC system equipped with a Smartline 1000 quaternary pump version 7603, manager 5000 version 7602, UV detector 2600 version 7605, dynamic mixing chamber version 1119-1 and Chrom Gate HPLC software 3.1.7. The Rheodyne injector was fitted with a 20 μL loop. The analytical separation was performed on a Eurosphere 100-5 C18 column (250 × 4.6 mm, 5 μm). The solvent system was a mixture of acetonitrile-water (25:75 v/v) containing 0.05% ortho-phosphoric acid to achieve maximum separation and sensitivity. This mobile phase was sonicated before use and was degassed before injection into HPLC. The flow rate was 1.0 mL/min and
the injection volume for all samples was 20 μL. The analyses were monitored at 282 nm for glaucine and 254 nm for quercetin and kaempferol. Each determination was carried out in triplicate. All chromatographic analysis was performed at ambient temperature. The chromatographic peaks of glaucine, quercetin and kaempferol in the sample solutions were confirmed by comparing their retention time and UV spectrum with those of the reference standard. Quantification was made according to the linear calibration curves of the standard compounds glaucine, quercetin and kaempferol.

2.5.1. Preparation of standard solutions and calibration curve

The stock solutions (100 μg/mL) of the alkaloid glaucine and the flavonoids quercetin and kaempferol as the standard compounds were prepared in methanol. The concentrations of 5, 10, 20, 30, 40 and 50 μg/mL for glaucine and 0.5, 1, 2, 4, 6 μg/mL for quercetin and kaempferol were prepared and stored at 4 °C. Different concentrations of standard solutions (X) were injected into the HPLC system in triplicate, and the average peak areas (Y) were calculated. Calibration curves were constructed by plotting peak areas versus concentrations of glaucine, quercetin and kaempferol and the linear regression equations were calculated.

2.6. Statistical analysis

All data were statistically analyzed using SPSS software (SPSS, Version 18 for Windows, SPSS Inc., Chicago, USA). For determining the significant differences at p < 0.05 level, the one-way analysis of variance (ANOVA) and Duncan’s multiple range test were performed. Analyzed data were presented as the mean ± standard error (Mean ± S.E).

3. Results and discussion

Interactions of NPs with plants are very important for evaluating their toxicity, transporting through a growth environment and penetration into plant tissues [37]. Based on different studies, NPs are capable of producing stress and generating excess ROS, which potentially affects the activities of lipids, carbohydrates, proteins, and DNA in plants [38]. However, the influence of NPs on secondary metabolites content in medicinal plants has been poorly studied. Thus, in the present study, we evaluated for the first time the influence of TiO2, Al2O3 and NiO NPs on secondary metabolites content, including the contents of total flavonoid, total alkaloid, glaucine, quercetin and kaempferol in endemic N. arvensis by HPLC method.

3.1. Total alkaloid content

In the present study, based on ANOVA analysis, there was a significant difference in the content of the total alkaloid of N. arvensis between the treatment and control groups at p ≤ 0.05. In all treatment groups, the total alkaloid content was significantly increased compared to the control groups except for the treatment groups of 50 mg/L of Al2O3 and TiO2 NPs, in which the level of total alkaloid was reduced (Figure 1). The highest total alkaloid content was observed at the treatment of 10 mg/L Al2O3NPs followed by 100 and 1000 mg/L NiONPs (Figure 1). Alkaloids are one of the major groups among the three main groups of natural plant products (terpenes, phenolic compounds, and alkaloids), which are influenced by different elicitors [11]. In the present study, different treatments of NPs led to an increase of the total alkaloid content compared to control groups except for the treatments of 50 mg/L of Al2O3 and TiO2 NPs (Figure 1). This increase was more than 50% in the treatments of 100–1000 mg/L Al2O3NPs and NiONPs. Similarly, in the study of Ghorbanpour et al. (2015), the increased alkaloid content in Hyoscyamus niger L. exposed to 20, 40 and 80 mg/L TiO2NPs (10–15 nm in size) was attributed to the greater accumulation of dry matter and increased alkaloid biosynthesis under treatment conditions [39]. Furthermore, the authors suggested that the key enzymes (i.e. potreacin-N-methyltransferase and hyoscyamine 6-β-hydroxylase) involved in the biosynthesis of tropane alkaloids may be affected in the treatment with NPs, which refers to the metabolic compatibility of treated plants in response to the harmful and side effects created by these NPs [39].

3.2. Total flavonoid content

The total flavonoid content of N. arvensis increased significantly (p < 0.001) upon exposure to all three nanoparticles compared to the control group (Figure 2). As seen in Figure 2, the highest amount of total flavonoids among all treatments was observed for the treatment of 100 mg/L NiONPs with a 2.5 fold increase compared to control. Also, the increased total flavonoid content was observed in Al2O3NPs treatment at concentrations of 50–2500 mg/L. Moreover, after exposure to TiO2NPs, the total flavonoid content enhanced 2.2 times at the concentration of 1000 mg/L compared to control. Flavonoids, as an important class of natural antioxidants, play a significant role in the removal of free radicals and the chelation of metal ions. Under biological and non-biological stresses such as drought, salinity, heavy metals, etc., which produces ROS and oxidative stress in plants and limits the efficiency of carboxylation, the activity of ROS detoxifying enzymes may be reduced in chloroplasts, in which case the plants ultimately increases the biosynthesis of ROS-removing flavonoids against these stresses [40]. Therefore, with the production of ROS in N. arvensis under stress to applied NPs in the present study by releasing metallic ions inside the plant, the plant increases the biosynthesis of flavonoids (Figure 2) to chelate the metal ions and prevent the formation of free radicals.

In the similar studies on Vigna radiata, Phaseolus vulgaris, Triticum aestivum, Lepinna minor and Sporodes polyrhiza treated with AgNPs (50 mg/L) and NiONPs (50 and 120 mg/L), the total flavonoids content was increased [41, 42, 43], but in the study of Javed et al. (2016) on Stevia rebaudiana exposed to zinc oxide nanoparticles (ZnONPs), it was increased at concentrations of 0.1 and 1 mg/L and decreased at higher concentrations of 10, 100 and 1000 mg/L [44]. In another study by Ghorbanpour (2015), increased the total flavonoid content was reported in Salvia officinalis treated with TiO2NPs (at concentrations of 0, 10, 50, 100, 200 and 1000 mg/L) compared to the control [45], that these results are accordance with the results of the present study.

3.3. Identification and quantification of glaucine, quercetin and kaempferol

To our knowledge, no phytochemical investigations on the alkaloid and flavonoid contents of N. arvensis have been conducted, and we report here for the first time. This study describes the quantification of the alkaloid glaucine and the flavonoids quercetin, and kaempferol by HPLC method in roots and shoots of N. arvensis growing in five groups: untreated group (as a control) and treated groups with TiO2NPs, NiONPs and Al2O3NPs at concentrations of 50, 100, 1000 and 2500 mg/L for 21 days. The method linearity was tested using the standard solutions of glaucine, quercetin, and kaempferol. The calibration curve of glaucine was linear in the range of 5–50 μg/mL, with a correlation coefficient of 0.998 and a linear equation of Y = 44155X – 1831 (Figure 3A). Also, the calibration curves of quercetin and kaempferol were linear in the range of 0.5–6 μg/mL, with the correlation coefficients of 0.997 and 0.998 and the linear equations of Y = 55721X – 10727 and Y = 65538X + 13096, respectively (Figure 3B).

Figure 4 shows HPLC chromatograms of standard samples of glaucine, quercetin and kaempferol at the highest concentration. A good separation was achieved at the retention time of 8.2 min for glaucine in root and shoot organs during the working day at a wavelength of 282 nm (Figure 4A). As shown in related chromatograms, the retention time of quercetin was in the range of 26–28 min and the retention time of kaempferol was achieved in the range 27–30 min in the root and shoot organs at a wavelength of 254 nm (Figure 4B and C).
3.3.1. Determination of glaucine content

Based on the analysis of variance in terms of the glaucine content, a significant difference at the level of $p < 0.001$ was observed between the various treatment groups and the control group in the shoots and the roots of *N. arvensis* under stress to three different types of studied NPs (Figure 5). In the shoots and roots of *N. arvensis* treated with NiONPs, the glaucine content was significantly increased compared to the control group. In the shoots, the highest glaucine content was recorded at a concentration of 1000 mg/L of NiONPs, so that its level was increased to 3.2 fold compared to the control group (Figure 5A). Also, in the root part, the glaucine content was increased at a concentration of 2500 mg/L of NiONPs by 2.5 fold increase against the control group. Among the different treatments, it was observed a decrease in the content of glaucine at a concentration of 50 mg/L NiONPs in the root part of the plant (Figure 5B).

The glaucine content in the shoot and root parts of the plant treated with Al$_2$O$_3$NPs also showed a significant increase compared to the control group, but a slight decrease was observed at a concentration of 1000...
mg/L. The highest level of glaucine in the shoots treated with Al\textsubscript{2}O\textsubscript{3}NPs was observed at a concentration of 2500 mg/L that was 36.8% higher than control. Also, the highest level of glaucine in the roots was observed at concentrations of 100 and 2500 mg/L with a 1.6 fold increase compared to the control group (Figure 5B). The content of glaucine was increased in the plants treated with TiO\textsubscript{2}NPs at concentrations of 2500 and 50 mg/L in the shoots and roots by 2.4 and 1.7 fold compared to the control group, respectively. Among different treatments of TiO\textsubscript{2}NPs at
the root part, a decreasing trend was observed in the glaucine content (Figure 5B), while its amount at the shoot part was reduced only at a concentration of 1000 mg/L compared to other treatments (Figure 5A). Accordingly, Shakeran et al. (2015) showed that AgNPs have an active role in the increased level of atropine in *Datura metel* [46]. Therefore, these applied NPs can increase the content of secondary metabolites owing to several reasons. Firstly, the NPs can act as a signal molecule to induce the production of plant secondary metabolites and physiological responses, but their mechanisms of action are not clear [47]. Secondly, the entrance of NPs into plant cells and their accumulation induce ROS generation, lipid peroxidation (by the accumulation of ROS) and increased malondialdehyde content and thus activate the plant antioxidant responses that one of them may increase the production of secondary metabolites [48, 49, 50, 51]. Thirdly, NPs have significant effects on the transcriptional regulation of specific genes and the activity of basic enzymes of biosynthetic pathways of alkaloids or are involved in the production of secondary metabolites [20, 52]. Furthermore, Jasmonate, as an important regulator of plant growth, stimulates several plant defense responses, for instance, the biosynthesis of secondary metabolites [48, 49, 50, 51]. Therefore, it seems that NPs may help in signal transduction pathways that promote the induction of jasmonate genes in cells under treatment [53].

Our results are accordance with the findings of Ghorbanpour et al. (2015), who showed that the hyoscyamine content was increased at the highest concentration of TiO$_2$NPs (80 mg/L), while the content of alkaloid scopolamine was enhanced at the lowest concentration (20 mg/L) [39]. Some studies have shown that TiO$_2$NPs in plants affect the nitrogen metabolism with improving the activity of some enzymes such as glutamate dehydrogenase and nitrate reductase and also increasing the growth and photosynthesis rate. In addition, these NPs increase the amount of protein and stimulate the expression of plant genes by enhancing nitrogen metabolism and its production [54]. Therefore, in our research, studied NPs probably increase the production of alkaloid glaucine by enhancing nitrogen metabolism.

In our study, the glaucine content was decreased against 1000 mg/L of TiO$_2$NPs and Al$_2$O$_3$NPs and 2500 mg/L of NiONPs. The decreased production of glaucine content may be due to the toxic effects of NPs on the mitotic index (genotoxic) and DNA or gene expression [55]. The increased time of treatment with Fe$_3$O$_4$NPs and their concentration resulted in a decline in the production of the alkaloid tropane [56]. These results suggest that these NPs can be applied as new, important and potent elicitors to increase the large-scale production of secondary metabolites in plant biotechnology.

### 3.3.2. Determination of quercetin and kaempferol contents

The successfully validated and established HPLC–UV method was applied for simultaneous quantification of the flavonol aglycones of quercetin and kaempferol in the shoot and root samples of *N. arvensis* under 21-days stress to NPs. Each analyte in the prepared samples was quantified using the peak area ratio according to the calibration curve of each standard. The results indicated that the applied treatments affected the quercetin and kaempferol contents in the shoot and root samples of *N. arvensis*. As shown in Figure 6A, the quercetin content in the shoot part was significantly increased upon exposure to all treatment groups over the control group except for treatments of 50 mg/L Al$_2$O$_3$NPs and 1000...
mg/L TiO₂NPs. The highest content of quercetin was observed significantly in shoots (0.97 mg/g) and roots (0.45 mg/g) treated with NiONPs at a concentration of 50 mg/L by 2.2 and 1.8 folds increase compared to the control group, respectively and then it was observed at the concentration of 1000 mg/L NiONPs (Figure 6). Furthermore, after treatment with Al₂O₃NPs at concentrations of 2500 mg/L in shoots and 1000 mg/L in roots, the quercetin content was increased 22% and 43%, respectively in comparison with the control group. Also, in the treatment with TiO₂NPs, the highest content of quercetin was recorded at concentrations 50 mg/L in shoots (Figure 6A) and 2500 mg/L in roots (Figure 6B) against the untreated group by 1.5 and 1.3 fold increase, respectively. As shown in Figure 6B, the reduction in quercetin content mostly occurred in the roots because the roots were in direct contact with the nanoparticles stress and thus are more susceptible to this stress.

According to ANOVA analysis of kaempferol content in N. arvensis under treatment with TiO₂NPs, Al₂O₃ and NiO NPs, there was a significant difference between the treatment and control groups (Figure 7). Under NPs stress the kaempferol content showed an irregular reduction in the shoots (Figure 7A) and roots (Figure 7B) compared to the untreated group. Of course, a remarkable increase in the content of kaempferol occurred in treatment with NiONPs at a concentration of 1000 mg/L in the root part with a 2.9 fold increase in comparison to the control group (Figure 7B). The lowest content of kaempferol was observed in the plant treated with Al₂O₃NPs in the roots and shoots at concentrations of 2500 and 50 mg/L, respectively.

Based on studies, the exposure of plants in the cell suspension culture to the elicitors, including different NPs, induces a cascade of signal transduction, which leads to the expression of different genes encoding the enzymes involved in the biosynthetic process of secondary metabolites [57].

The excessive production of free radicals, including superoxide, hydrogen peroxide, and hydroxyl in the plant cells following contact with NPs, can be another potential mechanism, which leads to increased production of secondary metabolites. According to Jobs et al. (1997), the production of hydrogen peroxide may alter the redox state of plant cells and act as a signaling molecule for the induction of biosynthetic pathways of secondary metabolites [58]. The contents of different classes of secondary metabolites, including phenolic compounds, flavonoids, saponins, iridoids, caffeic acid, and rosmarinic acid, have been enhanced as a result of increasing the content of hydrogen peroxide during exposure to carbon nanotubes, Al₂O₃ and NiO NPs [3, 55].

Moreover, in the study of Javed et al. (2016) on Stevia rebaudiana treated with ZnONPs, the stevial glycoside content was increased in 1 mg/L ZnONPs compared to control, but it was decreased at higher concentrations of 100 and 1000 mg/L. They stated that ZnONPs act as oxidative stress by releasing metallic ions or free radicals into the culture medium. Therefore, at a high concentration of NPs (1000 mg/L), an imbalance occurs between the antioxidant activity and the oxidative pressure, and hence the plant growth is disturbed and the amount of steviol glycoside is decreased [44].

Also, it has been reported that an increase in the aloin content of Aloe vera treated with TiO₂NPs until 48 h and its reduction after that may be related to the toxic effects of TiO₂NPs or its impacts on genes expression [18]. In addition, the enhancement of hypericin and hyperforin in plant exposed to zinc and iron oxide NPs, has been attributed to jasmonate hormone, which plays a significant and important role in stimulating hormone for plant defense responses, including the increased biosynthesis of secondary metabolites under different stress conditions, and NPs may play a significant role in the process of signal transduction that regulates the jasmonate producing genes in treated cells [53]. The effects of Ni on the content of hypericin and hyperforin were studied by Morch et al. [59]. In this study, the reduction of 15–21 folds in hypericin content and hyperforin below the detection limit compared to the control group attributed to the metabolic changes in the plant, including synthesis of organic compounds to detoxify the nickel and changes in absorption and accumulation of other important metals (such as molybdenum and iron) in plant metabolism, which may result in the inactivation of specific enzymes activity or biosynthetic processes of secondary metabolites [59]. These studies confirm the increased or decreased contents of the flavonoids quercetin and kaempferol in the shoot and root parts of N. arvensis treated with different NPs. Therefore, it has been shown that different nanoparticles at various concentrations and conditions can have different effects on plants by activation of particular mechanisms.

4. Conclusions

We analyzed for the first time the contents of the aporphine alkaloid glaucine and the flavonol aglycones quercetin and kaempferol in the root and the shoot extracts of Iranian species of N. arvensis using a simple HPLC method with UV detection. Furthermore, in the present study, we demonstrated the effects of different levels of three NPs (TiO₂, Al₂O₃, and NiO NPs) on the contents of total alkaloids and total flavonoids and as well as the content of glaucine, quercetin, and kaempferol. The comparative study showed increased production of total alkaloids, total flavonoids and glaucine content in the shoot and root parts compared to the control group. This increase could be a sign of antioxidant response of the plant against stress conditions caused by different NPs. Quercetin content was increased in the shoot and root parts exposed to all concentrations of NiONPs and higher concentrations of TiO₂ and Al₂O₃ NPs. Kaempferol content was decreased in treated plants due to the toxic effects of NPs. However, these results showed that some NPs at specific levels can be used as appropriate elicitors for in vitro production of secondary metabolites for medicinal applications of plants. NPs should be used with caution; they can alter the production of plant compounds thus affecting their medicinal value.

Declarations

Author contribution statement

A. Chahardoli: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
M. Modarresi: Performed the experiments; Contributed reagents, materials, analysis tools or data.
N. Karimi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.
S. Chahardoli: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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