RESEARCH ARTICLE

Neuroprotective Ability of Tobacco Stem Silver Nanoparticle on Rat PC-12 Cells

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Abstract

Aims: The present study was approached to determine the neuroprotective ability of synthesize silver nanoparticles (AgNPs) of stem of *Nicotiana tabacum* (TSAgNPs). **Materials and Methods:** Aqueous extract of tobacco stem was used as bioreducing agent to synthesize nanoparticles and was characterized by ultraviolet visible (UV-Vis) spectra scan, dynamic light scattering (DLS), and Fourier transformed infrared (FT-IR). Antioxidant content in TSAgNPs was determined by electron transfer assay (total flavonoid count, total phenolic content, and 1, 1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging), enzymatic biochemical assay (superoxide dismutase [SOD], catalase [CAT], and glutathione S-transferase [GST]), and non-enzymatic biochemical assay (reduced glutathione [GSH] content and malondialdehyde [MDA] content). Neuroprotective ability was determined by observing *in vitro* antioxidant activity of TSAgNPs on Rat PC-12 cells by exposing it to hydrogen peroxide as neurotoxic agent. **Statistical Analysis Used:** To estimate the accuracy of the experimental data, each experiment was performed in triplicates, and the result was expressed as the mean ± standard deviation of three replications. $P < 0.05$ was considered as statistically significant. **Results:** This was found from this study that by UV-Vis spectra scan has shown the maximum absorbance at 456 nm, whereas DLS has shown the size of TSAgNPs, i.e., 565.1 Dia(nm). FT-IR has confirmed the bioconjugate formation by giving intense bands at 3298.83, 2333.38, 1639.39, 1085.46, and 1038.83/cm wavenumber. Antioxidant content in TSAgNPs was found to be present, and it has shown the presence of flavonoid content, i.e., 74.85 ± 0.22 mg QE/g of TSAgNPs, phenolic content, i.e., 502 ± 0.06 mg QE/g of TSAgNPs and DPPH free radical scavenging found to be 60% of inhibition. Enzymatic and non-enzymatic biochemical assay was determined and has shown the presence of maximum specific enzyme activity in TSAgNPs. *In vitro* antioxidant activity, i.e., SOD, CAT, GST, GSH content, and MDA content in Rat PC-12 cells was found to be maximum as the volume of TSAgNPs increased in cells against the neurotoxic agent, and this observation reveals the presence of neuroprotective ability. **Conclusions:** This can be concluded from the present study that TSAgNPs can be used as a natural herbal remedy to treat the neurological disorders as a neuroprotective agent and to make use of waste material, i.e., stem of tobacco for therapeutic purposes.

Key words: Catalase, glutathione S-transferase, lipid peroxidation, neuroprotection, *Nicotiana tabacum*, Rat PC-12 cells, silver nanoparticles, stem, superoxide dismutase

INTRODUCTION

Nanotechnology has become status as one of the research endeavors of the 21st century as scientist harness properties of atomic and molecular assemblage built at the nanometer scale. Nanoparticles have been produced by physical, chemical, and mechanical means, where disadvantages have been observed such as increased size, high energy, and capital intensiveness.[1] As far as "green" environment-friendly is concerned, a process involving chemistry and chemical technologies has become popular nowadays. These technologies have shown much needful results of worldwide problem associated with environmental concerns.[2,3] In every phase of science along with engineering, metallic nanoparticles are being utilized that has explored their new dimension for their respective worth. An increasing awareness toward green chemistry and synthesis of metal nanoparticles has developed environment

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friendly, where green synthesis of nanoparticles using plant extracts has shown economical, energy coefficient, and cost-effective. Silver which has long been recognized as inhibitory efficacy against pathogenic bacterial cells has proved to be ointment and cream nanoformulation that has prevent infection of burns and wounds.[4] Silver nanoparticles (AgNPs) have also shown its anticancer activity as a positive response against cancer cells.[5] Herbal extracts have shown a therapeutic effect of their bioactive compounds as antimicrobial, anti-inflammatory, antioxidant, anticancer, and antidiabetic activities. Extracts of plants are used for the synthesis of metallic nanoparticles such as AgNPs by bioreduction method. Characterization of synthesis AgNPs has involved the use of technologies such as scanning electron microscope, transition electron microscopy, and dynamic light scattering (DLS).[6] Various techniques have been taken under to synthesize nanoparticles such as sugars, biodegradable polymer, plant extract, and microorganism as reductants and capping agents.[7] Microorganism has been used to synthesize nanoparticles, but it was found that synthesis becomes slower as compared to plant-mediated synthesis.[7] Herbs have shown the interaction of metal ions to form metallic nanoparticles. As a source, the medicinal plant has also shown advantages such as cost-effectiveness, non-toxic, and eco-friendly agent. Biosynthesis of nanoparticles by medicinal plant extracts are currently under exploitation.[8]

Nicotiana tabacum, commonly known as tobacco, is a perennial herbaceous plant which belongs to the family of Solanaceae.[9] 20% of tobacco resources are discarded as waste such as its stem that has no use, but in the recent studies, proved that it has antioxidant and antimicrobial activity in its aqueous and methanolic extract.[10] Leaves of *N. tabacum* that is basically discarded have valuable bioactive compound such as polyphenols. Antibacterial activity was also found to be present in nicotine associated with zinc against both Gram-positive bacteria as well as Gram-negative bacteria.[11] Stem of *N. tabacum* has shown therapeutic properties by revealing its antibacterial and antioxidant activities.[12] Stem of *N. tabacum* has also shown the presence of flavonoids and phenolic content in its aqueous extracts.[12]

The purpose of the present study was to synthesize AgNPs from the stem of *N. tabacum,* i.e., tobacco stem AgNPs (TSAgNPs). UV spectra scan, DLS, and Fourier transformed infrared (FT-IR) spectra were used to characterize the nanoparticles. Antioxidant content of TSAgNPs was determined on the basis of antioxidant activity of nanoparticles, which were observed by electron transfer, enzymatic biochemical, and non-enzymatic biochemical assay. The neuroprotective ability of TSAgNPs was measured by giving neurotoxic shock to Rat PC-12 cells and then observing the enzymatic and non-enzymatic biochemical assay. The present study also provides rational use of these nanoparticles for therapeutic use against neurological disorders.

MATERIALS AND METHODS

Plant sample

Stems associated with leaves were collected of *N. tabacum* from the Khari Baoli, Kucha Challan, Chandni Chowk, Delhi, and kept at Amity Institute of Biotechnology, Amity University Uttar Pradesh. Stems were separated out from the leaves and washed with distilled water to remove dirt from it. Stems were dried in a shaded area at room temperature and used throughout the project.

Neuronal cells

Rat PC-12 cells were obtained from NCCS, Pune, and were subcultured in fresh DMEM media and used throughout the project.

Preparation of aqueous extract

Dried stem was crushed by an ordinary grinder to make a fine powder. 10 g of powder was dissolved in 100 ml of double distilled water. It was boiled in a water bath at 100°C for 30 min. The conical flask was covered with cotton plugs to avoid evaporation. Aqueous extract was filtered with muslin cloth and then with filter paper twice. Prepared aqueous extract was stored at 4°C and used for the synthesis of TSAgNPs.[12]

Green synthesis of AgNPs

TSAgNPs were formed by bioreduction method. 30 ml of extracts were preheated and 2 ml of 1mM of silver nitrate has been poured into it. Solutions were kept in the dark chamber to avoid the photoactivation of silver nitrate. The solution has been incubated for 24 h for the synthesis of nanoparticles. Capping of TSAgNPs has been done by 5% sodium alginate to prevent it from agglutination. Stabilization and destabilization have been observed by ultraviolet visible (UV-Vis) spectra scan. Day-by-day, their scan has been estimated to observe the maximum absorption at a particular wavelength in the range of 200–800 nm.

Characterization of nanoparticles

UV-Vis spectral analysis was done using Shimadzu spectrophotometer (UV-1800, Japan). UV/Vis absorption spectrophotometer with a resolution of 1 nm between 200 and 800 nm was used. One milliliter of the sample was pipetted into a cuvet and subsequently analyzed at room temperature. DLS (Spectroscatter 201) was used to determine the average size of synthesized TSAgNPs. FT-IR spectra were recorded on Perkin Elmer 1750 FT-IR spectrophotometer.[6]

Antioxidant Assay

Antioxidant capacity synthesized TSAgNPs of stem of *N. tabacum* has been determined by three different methods, i.e., electron transfer assay, enzymatic biochemical, and non-enzymatic biochemical assay which were determined in nanoparticles:

A. Electron transfer assay: Total flavonoid content (TFC), total phenolic content (TPC), and 1, 1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging have been assayed to determine the oxidants which were reduced by transfer of electron from an antioxidant (oxidized).
   i. TFC
      A number of flavones that were present in TSAgNPs of stem have been attached to the silver ions while synthesis of TSAgNPs, which were determined by aluminum chloride spectrophotometric method. About 0.1 ml of TSAgNPs or quercetin standard 10–100 µg/ml, 1.5 ml of methanol, 0.1 ml aluminum chloride (10%), 0.1 ml potassium acetate (1M), and 2.8 ml of distilled water were added and mixed well. Sample blank was prepared by replacing sample with distilled water or solvent, and absorbance was measured at 417 nm after the incubation of 30 min in the dark. Standard calibration plot was made to determine the concentration of flavonoids in the TSAgNPs. The concentrations of flavonoids in the TSAgNPs were calculated from the calibration plot and were expressed in mg QE/g of TSAgNPs.[12,13]
   ii. TPC
      Flavonoids are polyphenolic compounds, so the TPC has been determined by Folin–Ciocalteau method to quantify the polyphenolic flavonoids. 0.1 ml of TSAgNPs or quercetin standard (10–100 µg/ml) and 0.1 ml of Folin–Ciocalteau reagent (0.5N) was added and incubated at room temperature for 30 min. About 2.5 ml of 20% saturated sodium carbonate was added into the solution and further incubated for 30 min. After incubation, the absorbance was measured at 760 nm against blank reagent. The standard calibration plot was made to determine the concentration of polyphenolic component in the TSAgNPs were calculated from the calibration plot and were expressed in mg QE of phenol/g of TSAgNPs.[13,14]
   iii. DPPH free radical scavenging
      Free radical scavenging has been assayed in TSAgNPs of stem of *N. tabacum*. The antioxidant capacity of nanoparticles was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were performed out according to the standardized protocol.[13] 3.8 ml of methanol was taken as blank, whereas 3 ml of methanol added in 0.3 ml of 0.4 mM DPPH solution was taken as control. Reaction mixture was prepared by taking 3 ml of methanol mixed well with volumes TSAgNPs in the ranges (50–300 µl) and 0.3 ml of 0.4 mM DPPH solution. A solution such as blank, control, and reaction mixture was allowed to incubate in the dark for 30 min. The color of the reaction mixture faded as compared to the control, and the reduction is observed by the decrease in the absorbance at 517 nm. The results were expressed in percentage of inhibition using formula. The results were compared with the positive control, i.e., standard quercetin. The percentage inhibition of the DPPH radical was measured using the following formula.[15]
      Percentage of inhibition = [(Absorbance of control− Absorbance of reaction mixture)/absorbance of control]×100

B. Enzymatic biochemical assay: Enzymatic biochemical assay was determined by catalase enzyme activity (CAT), superoxide dismutase activity (SOD), and glutathione S-transferase activity (GST) in the TSAgNPs by standardized protocol. CAT enzyme activity was expressed in enzyme activity as µ moles of H_2O_2 oxidized per mg protein.[16] SOD activity was defined as 1 unit enzyme concentration for 50% inhibition at an absorbance of 560 nm of chromogen produced in 1 min under assay. SOD activity was expressed in specific enzyme activity as unit of SOD per min per mg of protein.[17] GST activity was determined in TSAgNPs to observe the detoxification of compounds that were involved in the reducing free radical damages in blood cells. GST assay was also a specific enzyme activity that was based on the glutathione conjugate to 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate and was measured at absorbance of 340 nm. Enzyme specific activity was expressed in µmoles of CDNB-reduced glutathione (GSH) conjugate formed per min per mg of protein.[18]

C. Non-enzymatic biochemical assay: Glutathione and malondialdehyde content (MDA) were determined to assay the non-enzymes. For MDA, lipid peroxidations of thiobarbituric acid reactive substances were estimated. This was determined by breaking down of polyunsaturated fatty acids where lipid peroxidation levels were expressed in nanomoles of MDA formed per gram of TSAgNPs by observing spectrophotometrically at 512 nm.[19] Glutathione content was estimated in TSAgNPs using dithiobis nitrobenzoic acid and expressed in µg per mg of protein.[20]

Quantification of protein

Protein was quantified in the 100 µl of TSAgNPs of the stem of *N. tabacum* by Lowry's method using Folin–Ciocalteau Reagent.[21]

Neuroprotective ability on Rat PC-12

Neuroprotective efficacy of TSAgNPs on Rat PC-12 cell was observed by giving neurotoxic shock to Rat PC-12 cells

using 0.5 mM hydrogen peroxide. After subculturing of Rat PC-12 cells in RPMI media, 10^6 cells/well were plated in duplicates for each sample in microtiter plates. The plates were incubated for 48 h in CO_2 incubator at 37°C. First set of wells contained only cells as positive control. Second set contained cells + 0.5 mM hydrogen peroxide as negative control. Other sets of wells contained cells + 0.5 mM hydrogen peroxide + 2, 5 and 10 µl of TSAgNPs. Results were obtained on the determination of enzymatic and non-enzymatic biochemical assay.[16]

Statistical evaluation

To estimate the accuracy of the experimental data, each experiment was performed in triplicates, and the result was expressed as the mean ± standard deviation of three replications. $P < 0.05$ was considered as statistically significant.

RESULTS

Synthesis of TSAgNPs

The purpose of the present study was to investigate the neuroprotective ability of synthesize TSAgNPs by determining its antioxidant content and *in vitro* antioxidant activity. Aqueous extract of stem of *N. tabacum* has been prepared and AgNPs of it synthesized by bioreduction method. TSAgNPs have been formed by drop by drop method and capping has been done by sodium alginate to prevent it from agglutination. Stabilization and destabilization were observed by UV-Vis spectra scan, and it has revealed the lifespan of 3 months, as no agglutination was found in between 3 months.

Characterization of TSAgNPs

UV-Vis spectra scan was done to determine the maximum absorption at particular wavelength, i.e., $\lambda_{max.}$ 456 nm found to be wavelength where nanoparticles have shown maximum absorption as shown in Figure 1. DLS was done to reveal the size of TSAgNPs, i.e., 565.1 Dia (nm) with 100% intensity and width of 119.1 nm as given in Figure 2. FT-IR spectra of TSAgNPs were done to reveal the bioconjugate formation, where intense bands were found at 3298.83, 2333.38, 1639.39, 1085.46, and 1038.83/cm wavenumber as depicted in Figure 3. Potential biomolecules of tobacco stem found to be responsible for reducing the chloroaurate ions.

Antioxidant content

Synthesize TSAgNPs were evaluated for the determination of antioxidant assay. Different methods have been undertaken to observe the oxidizing efficacy of these synthesized TSAgNPs. Electron transfer, enzymatic, and non-enzymatic biochemical assays were evaluated in TSAgNPs. Electron transfer-based assay: Based on the transfer of electron, i.e., probe (oxidant) is reduced by transfer of an electron from an antioxidant (oxidized). The color change of the probe by oxidation is proportional to the amount of antioxidant.

A. TFC: This was done to estimate the number of flavones on TSAgNPs. Quantification of flavonoid was done by the aluminum chloride spectrophotometric method.

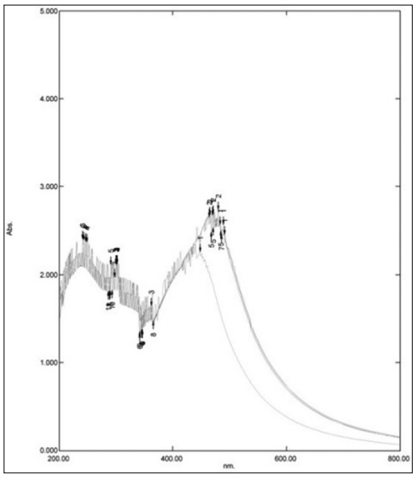


Figure 1: Ultraviolet-visible spectra scan of synthesized silver nanoparticles from the stem of *Nicotiana tabacum*

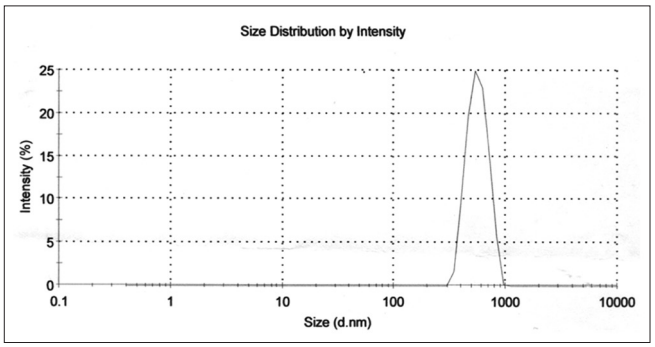


Figure 2: Dynamic light scattering of synthesized silver nanoparticles from the stem of *Nicotiana tabacum*

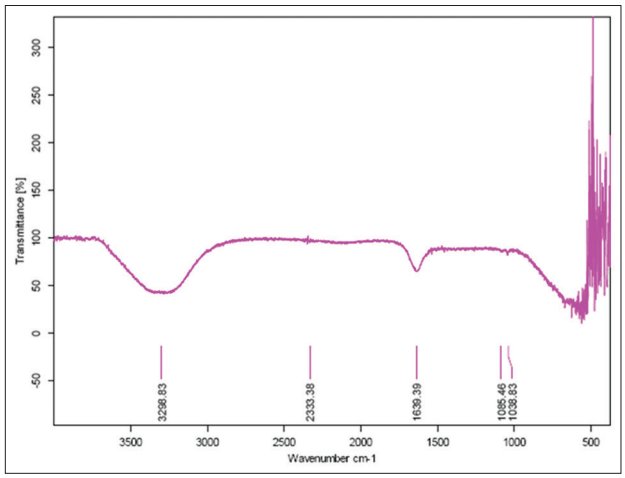


Figure 3: Fourier transformed infrared of synthesized silver nanoparticles from the stem of *Nicotiana tabacum*

Using a standard plot of quercetin (y = 0.0078x + 0.068, R^2 = 0.8524) as shown in Graph 1, the flavonoid content was found to be 74.85 ± 0.22 mg QE/g of TSAgNPs.

B. TPC: Phenolic content in the nanoparticles of stem of *N. tabacum* was determined by the Folin–Ciocalteau method. By standard curve of quercetin (y=0.001x−0.015, R^2 = 0.962) as shown in Graph 2. Synthesized TSAgNPs have shown the presence of phenolic content, i.e., 502 ± 0.06 mg QE/g of TSAgNPs.

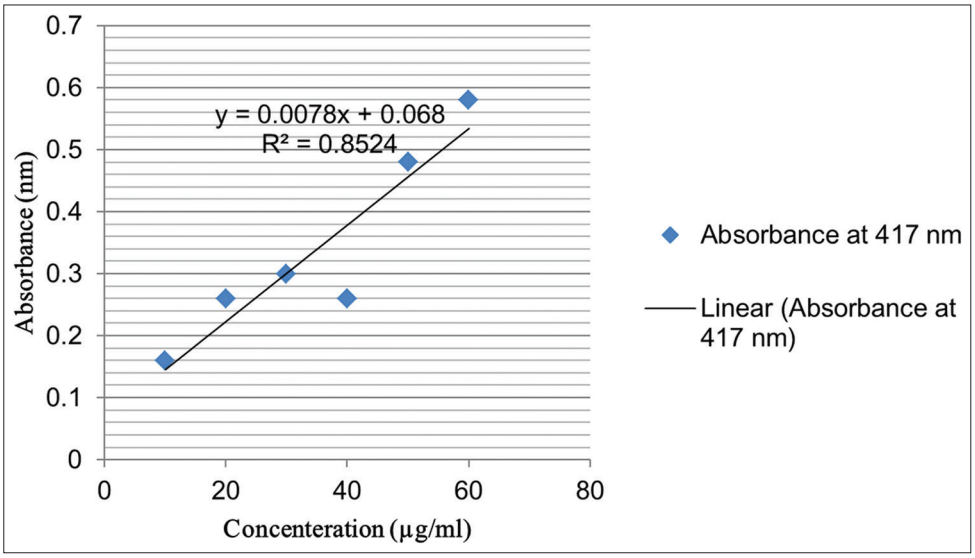
C. DPPH free radical scavenging assay: In the reaction mixture, antioxidant compound which was present in the sample donating a hydrogen atom because of which radical comes in a reduced form that was generally determined by the loss of color. As compared to standard quercetin, 60% of inhibition was found to be present in the TSAgNPs, whereas 70% was of quercetin as given in Figure 4.

Enzymatic assay

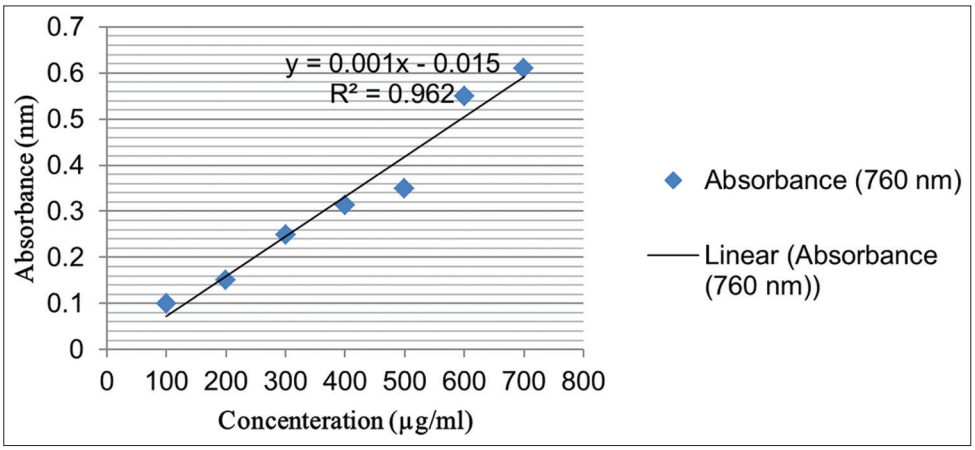
Antioxidant enzymatic activities of TSAgNPs were determined by SOD, CAT, and GST activity. TSAgNPs were evaluated in the ranges, i.e., 50–300 µl. It was found that maximum SOD activity was found to be present in 80 µl of TSAgNPs, i.e., 3.065 ± 0.03 unit/min/mg of protein, where lowest enzymatic activity found to be present in 300 µl of TSAgNPs, i.e., 0.5396 ± 0.3 unit/min/mg of protein as shown in Figure 5. As far as the CAT-specific enzyme activity was concerned, 200 µl of TSAgNPs has shown maximum enzymatic activity, i.e., 5.678 ± 0.13 µ moles of H_2O_2 consumed/min/mg of protein, whereas 50µl of TSAgNPs has shown lowest enzyme activity, i.e., 0.126 ± 0.05 µ moles of H_2O_2 consumed/min/mg of protein. From the Figure 5, maximum GST enzymatic activity was found to be present in 100 µl, i.e., 4.48 ± 0.04 µ moles of CDNB-GSH conjugate formed/mg of protein, whereas lowest amount of enzymatic activity found to present in 50 µl of TSAgNPs, i.e., 3.174 ± 0.02 µ moles of CDNB-GSH conjugate formed/min/mg of protein.

Non-enzymatic biochemical assay

Glutathione content was found to be present in the TSAgNPs in its increasing order of volumes from its



Graph 1: Quercetin standard curve for total flavonoid content



Graph 2: Quercetin standard curve for total phenolic content

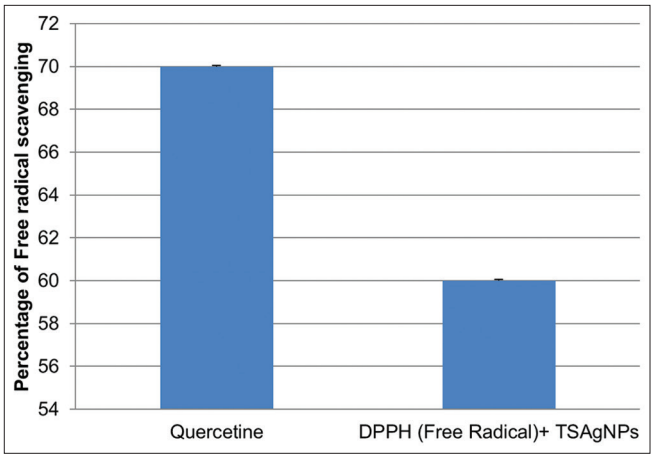


Figure 4: 1, 1-diphenyl-2 picrylhydrazyl free radical scavenging of synthesized silver nanoparticles from stem of *Nicotiana tabacum*

50 to 300 µl. 300 µl has shown the maximum content, i.e., 193.60 ± 0.45 µg of glutathione/mg protein as shown in Figurer 5. For lipid peroxidation, MDA content was determined in TSAgNPs, and it has shown the increase in the content from its 50 to 300 µl. It has shown MDA content that is 0.089±0.05 µ moles/mg of protein in 50 µl of TSAgNPs, whereas maximum MDA content found to be present in 300 µl, i.e., 0.109 ± 0.03 µ moles/mg of protein.

Neuroprotective ability of TSAgNPs on Rat PC-12 cells

This was the first attempt to determine the neuroprotective efficacy of synthesize TSAgNPs on rat PC-12 by analyzing its enzymatic and non-enzymatic biochemical assay in *in vitro* conditions. Rat PC-12 cells were exposed

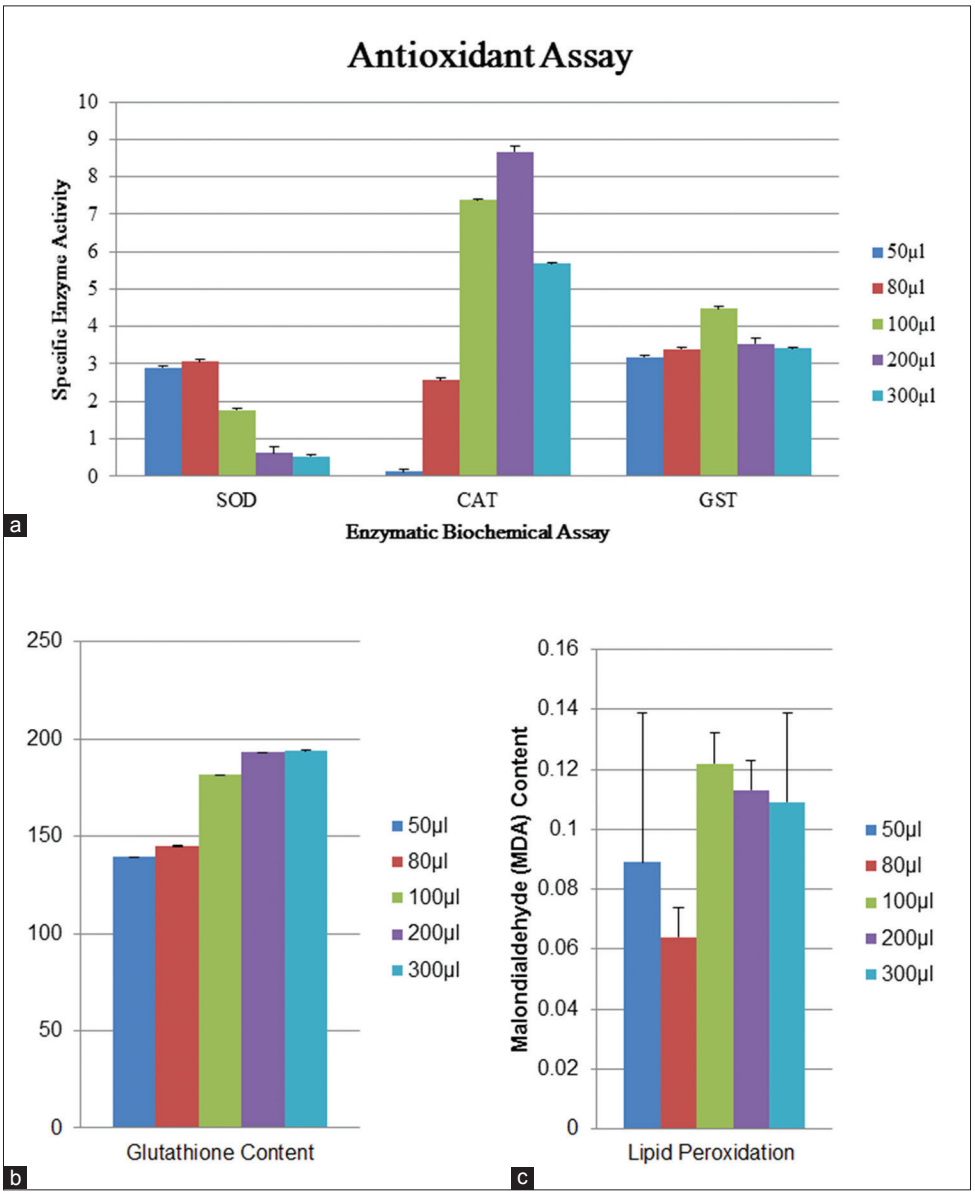


Figure 5: (a-c) Enzymatic and non-enzymatic biochemical assay of synthesized silver nanoparticles from the stem of *Nicotiana tabacum*

to neurotoxic shock with the help of 0.5 mM hydrogen peroxide. Cells were allowed to treat with the TSAgNPs in different volumes, i.e., 2, 5, and 10 µl against those that were exposed with 5 µl of 0.5 mM hydrogen peroxide. From the above study, it was found that SOD activity in 10 µl of TSAgNPs has shown maximum specific enzyme activity, i.e., 587.004 ± 0.1 unit/min/mg of protein, whereas compared to untreated cells, which has shown 507.131 ± 0.07 unit/min/mg of protein and treated with hydrogen peroxide, i.e., 482.40 ± 0.04 unit/min/mg of protein as given in Figure 6. CAT enzyme activity was also found to be present in 10 µl of TSAgNPs with specific enzyme activity of 270.68 ± 0.05 µ moles of H_2O_2 consumed/min/mg of protein against 5 µl of 0.5 mM hydrogen peroxide, whereas compared to the treated cells with 0.5 mM hydrogen peroxide, it has shown enzyme activity with 46.27 ± 0.034 µ moles of H_2O_2 consumed/min/mg of protein as shown in Figure 7. To determine the detoxification of TSAgNPs, GST activity was also determined in the treated and untreated cells with TSAgNPs. GST was found to be maximum in the 10 µl of TSAgNPs, i.e., 34.23 ± 0.14 µ moles of CDNB-GSH conjugate formed/mg of protein, whereas compared to the treated with free radicals one, it has shown 16.48 µ moles of CDNB-GSH conjugate formed/mg of protein as shown in Figure 8. For lipid peroxidation, MDA content was also determined in the treated cells and has shown its content level lowest in 10 µl of TSAgNPs in the cells against 5 µl of 0.5 mM hydrogen peroxide, i.e., 5.38 ± 0.03 µ moles/mg of protein, whereas treated with 2 µl of TSAgNPs has shown 1.35 ± 0.12 µ moles/mg of protein as shown in Figure 9. Glutathione content was also determined in the treated cells with nanoparticles, and it has shown maximum glutathione content in 10 µl of TSAgNPs, i.e., 62590.90 ± 0.36 µg of glutathione/mg protein as compared to those cells that were treated with 5 µl of 0.5 mM hydrogen peroxide, it has shown 34072 ± 0.09 µg of glutathione/mg protein as given in Figure 10.

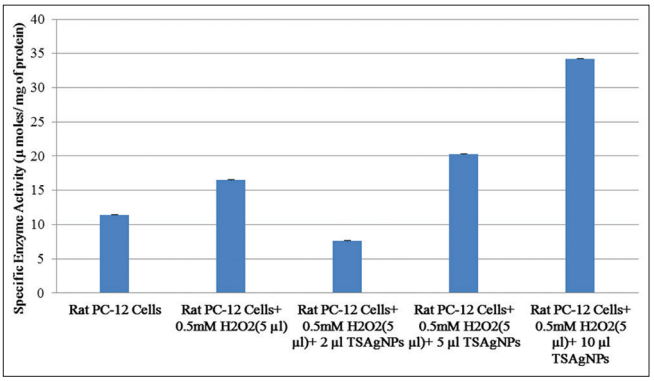


Figure 8: Glutathione S-transferase enzyme activity in Rat PC-12 after treated with tobacco stem silver nanoparticles against free radical

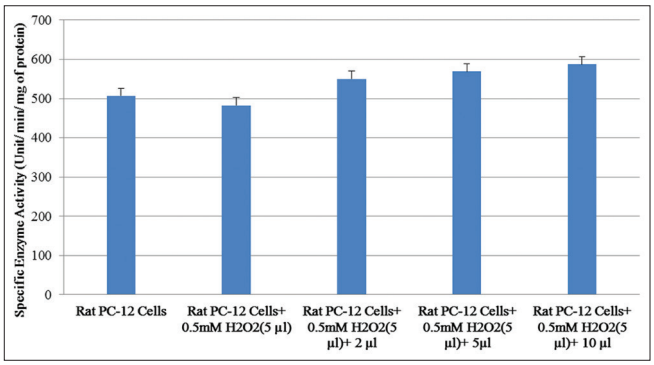


Figure 6: Superoxide dismutase enzyme activity in Rat PC-12 after treated with tobacco stem silver nanoparticles against free radical

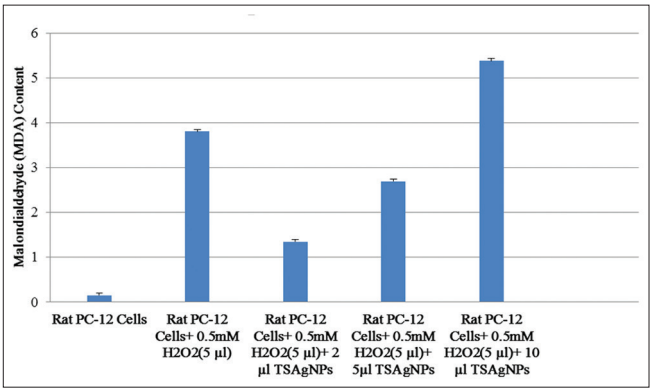


Figure 9: Lipid peroxidation in Rat PC-12 after treated with tobacco stem silver nanoparticles against free radical

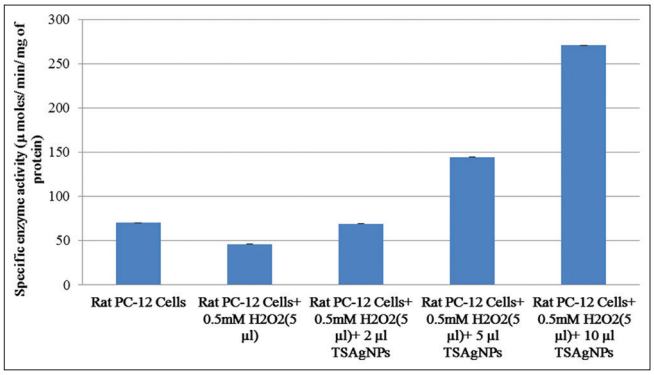


Figure 7: Catalase enzyme activity in Rat PC-12 after treated with tobacco stem silver nanoparticles against free radical

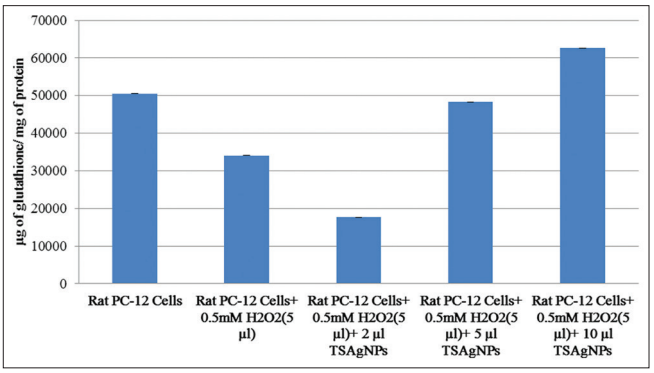


Figure 10: Glutathione content in Rat PC-12 after treated with tobacco stem silver nanoparticles against free radical

DISCUSSION

The purpose of the present study was to evaluate the neuroprotective ability of synthesize AgNPs of stem of *N. tabacum*. Reactive oxygen species leads to cellular damage and inflammation of the tissue. This plays a major role in cellular senescence paving way to neural cells death such as hydrogen peroxide or dopamine. In the previous studies, antimicrobial and antioxidant activity of polyphenolic flavonoids from stem of *N. tabacum* has already been studied. This literature quotes provoke us to synthesize AgNPs of stem of *N. tabacum* and evaluate neuroprotective efficiency on Rat PC-12 cells. Neuroprotective ability was determined on the basis of both *in vitro* antioxidant activity on Rat PC-12 cells and antioxidant assay of nanoparticles. Enzymatic biochemical assay and non-enzymatic biochemical assay were both determined in cells as well as in nanoparticles. TSAgNPs were synthesized by drop by drop method, and characterizations were done by UV-spectra scan, DLS, and FT-IR. FT-IR of TSAgNPs has shown the presence of C-H stretch at 3298.83/cm, whereas nitriles carbenes found to be present at 2333.38/cm. As far as double bond structures were concerned, they were found to be present at 1639.39/cm compared to the previous studies.[22] Antioxidant assays were determined in TSAgNPs and have shown the relevance of it's as flavonoids and phenolic content. This was found to be maximum in the nanoparticles, i.e., 74.85 ± 0.22 mg QE/g of TSAgNPs, as compared to the previous studies, flavonoids were found to be 12.5 ± 0.1322 mg QE/g of extract in its ethanolic extract of tobacco stem.[13] DPPH free radical scavenging assay was also determined on TSAgNPs, and it has shown the 60% of inhibition. SOD, CAT, and GST enzyme activity were also determined in the TSAgNPs and have shown maximum SOD-specific enzyme activity in 80 µl, i.e., 3.065 ± 0.03 unit/min/mg of protein as compared to the previous studies aqueous extract of tobacco stem has shown 2.866 ± 0.152 unit/min/mg of protein.[10] Maximum CAT enzyme activity was also found to be present in 200 µl with specific enzyme activity, i.e., 5.678 ± 0.13 µ moles of H_2O_2 consumed/min/mg of protein, whereas compared to the previous studies, 2.226 ± 0.289 13 µ moles of H_2O_2 consumed/min/mg of protein found to be present in aqueous extract of tobacco stem.[10] 13.017 ± 0.525 µ moles of CDNB-GSH conjugate formed/min/mg of protein found to be present in the aqueous extract of tobacco stem[10] as compared to the present studies 4.84 ± 0.04 µ moles of CDNB-GSH conjugate formed/min/mg of protein found to be present in the TSAgNPs.

Lipid peroxidation found to be low at 50 µl, i.e., 0.089 ± 0.05 µ moles/mg of protein, whereas compared to the previous studies that tobacco stem aqueous extract has shown the maximum MDA content. GSH content found to be maximum in TSAgNPs. *In vitro* antioxidant activity of synthesize TSAgNPs on neuronal cell i.e., Rat PC-12 cells has shown the presence of neuroprotective ability against neurotoxic shock i.e., hydrogen peroxide. Enzyme and non-enzyme biochemical levels were studied in both the treated and non-treated cells. It was found from the present study that SOD, CAT, and GST levels were found to be in increasing order as to show that while increasing the volume of TSAgNPs in cells against 0.5 mM of hydrogen peroxide can inhibit the free radicals. Both MDA content and GSH content levels were determined and compared to the previous studies.

CONCLUSION

From the above study, the authors would like to conclude that synthesize AgNPs of stem of *N. tabacum* has shown the presence of antioxidant activity. *In vitro* antioxidant activity of TSAgNPs has confirmed its neuroprotective ability against neurotoxic shock. These results provoke us to use it as a natural herbal remedy for neuroprotection as they can scavenge the free radicals. Further studies require the purification of secondary metabolites from stem of *N. tabacum* and conjugation of these metabolites with respective metal ions to evaluate their anticancer, anti-inflammatory, and many more pharmaceutical uses.

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