Toxicology Reports 8 (2021) 888–895

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Synthesis, characterization & evaluation of *venom neutralization* potential of silver nanoparticles mediated *Alstonia scholaris* Linn bark extract

Rituparna Ghosh [a], Sumana Sarkhel [a,*], Kanchan Saha [a], Poulami Parua [a], Upasana Chatterjee [b], Koushik Mana [c]

[a] Department of Human Physiology, Vidyasagar University, Paschim Midnapore, 721102, West Bengal, India
[b] Department of Microbiology, Vidyasagar University, Paschim Medinipur, 721102, West Bengal, India
[c] Department of Human Physiology with Community Health, Vidyasagar University, Paschim Medinipur, 721102, West Bengal, India

ARTICLE INFO

Edited by Dr. A.M Tsatsaka

Keywords:
Silver nanoparticles
Alstonia scholaris bark
Venom neutralization

ABSTRACT

Objective: The *venom neutralization* potential of silver nanoparticle(AgNP-AS) mediated bark extract of *Alstonia scholaris Linn* R.Br was investigated in the study.
Methods & materials: AgNP-AS was synthesized with respect to optimal temperature, pH of extract. UV–vis, FT-IR, XRD, TEM, SEM studies were used to characterize silver nanoparticles of *Alstonia scholaris* Linn(AgNP-AS). The potential of AgNP-AS in neutralization of venom lethality, rise in myotoxicity markers(LDH) and proinflammatory cytokines(IL6, TNFα) were evaluated in animal models.
Results: AgNP-AS was synthesized optimally with $AgNO_3$ (2 mM); extract concentration, 0.2 gm/l (1% w/v); extract (pH 9) and optimal temperature (40 °C). The colour change and synthesis of AgNP-AS was validated by UV–vis analysis at 432 nm. Transmission electron microscopy of AgNP-AS showed that the particle size for AgNP-AS was 14 nm-20 nm. FT-IR revealed peaks at 3445 cm^{-1}, 1646 cm^{-1}, 1346 cm^{-1} and 1108 cm^{-1}. From the dynamic light scattering studies the hydrodynamic diameter (115.87 nm) and zeta potential(-29.8 mV) were estimated. The EDAX exhibited a peak for silver validating that the synthesized silver was pure. The bio-synthesized (AgNP-AS) could significantly neutralize *Viper russelli* venom(VRV) induced rise in serum lactate dehydrogenase(LDH) and proinflammatory cytokines(IL6, TNFα) in animal models.
Conclusion: The culmination of nanotechnology with herbal medicine might endow with a really constructive tool in coming up with future drugs with fewer toxicity.

1. Introduction

Nanoparticles(NP) are defined as particles ranging from 10−100 nm. Silver nanoparticles are synthesized for their distinctive properties including shape, size dependent optical, antimicrobial and electrical properties. Nanoparticles(NP) have various applications including antimicrobial applications, biosensors etc. [1]. Synthesis of nano-particles using plants provide a large scale alternative production of nanoparticles(NP) [2]. Silver inhibits the growth of several bacterial strains. AgNP has been synthesized by using the plant broth from a wide variety of plants such as *Bacopa monnieri* [3], *Catharanthus roseus* [4] and *Coccinia grandis* [5]. Silver nano-particles possess bacteriocidal properties. The efficacy of nanoparticle production and incorporation of silver has facilitated to study the mechanistic aspects of anti-microbial, antiviral and anti-inflammatory potential of silver nanoparticles(NP).

Alstonia scholaris (L.) R.Br. belongs to the family *Apocynaceae*. It has been reported that the bark is used in ethnomedicine [6]. *Alstonia scholaris* L has been reported to possess antimicrobial potential [7]. Historically the plant has been used in ethnomedicine for treatment of chronic respiratory diseases.The leaf extract of the plant is used in Chinese medicine for treatment of cold and cough. The alkaloids present in the plant has therapeutic potential as anti-inflammatory and analgesic agent. The study describes the venom neutralization *potential* of silver nanoparticles based *Alstonia scholaris* Linn bark extract in *in vivo* models

Abbreviations: AAS, aqueous *Alstonia scholaris* Linn bark extract; AgNP-AS, silver nanoparticle of *Alstonia scholaris* Linn; $AgNO_3$, silver nitrate; DLS, dynamic light scattering; nm, nanometer; NP, nanoparticles; VRV, *Vipera russelli* Venom; *bw*, body weight; *i.v*, intravenous.

* Corresponding author.
E-mail addresses: rituparnaghosh0491@gmail.com (R. Ghosh), sumana.sarkhel@yahoo.in (S. Sarkhel), kanchansaha31@gmail.com (K. Saha), poulamiparua1993@gmail.com (P. Parua), upasana.chatterjee85@gmail.com (U. Chatterjee), koushik.lifescience@gmail.com (K. Mana).

https://doi.org/10.1016/j.toxrep.2021.04.006
Received 8 February 2021; Received in revised form 1 April 2021; Accepted 16 April 2021
Available online 19 April 2021

Table 1
Group allocation for Viper venom neutralization studies.

Groups (n = 6)	Treatment with doses(*i.v*)
Group-I	Saline control(0.9 % NaCl)
Group-II	Venom treated (VRV) (1 μg)
Group-III	Venom(1 μg) + AAS extract(200 mg/kg *bw)*
Group-IV	Venom (1 μg) + AgNP-AS(10 mg/kg *bw*)
Group-V	Venom (1 μg) + AVS (2 mg/mL)(50 μL)

thereby providing a substitute for traditional antiserum therapy.

2. Methods & materials

2.1. Chemicals

Sodium chloride (NaCl)(Sigma, India), lactate dehydrogenase kit (Sigma-Aldrich, India), IL6 and TNFα(Thermofisher Scientific, USA) were obtained from enlisted suppliers of the University.

2.2. Venom & antiserum

Venom in the lyophilized form was obtained from Calcutta Snake Park and preserved in dessicators at 4 °C in amber coloured glass vials and expressed as dry weight (mg/mL;*w/v*).

Snake venom antiserum I.P (batch no. 4066016) was obtained from VINS Bioproducts Limited, Hyderabad, India

2.3. Animals

Swiss albino mice(20 ± 2)g were procured from enlisted supplier of Vidyasagar University. Animal based experiments were ratified by Department of Human Physiology; Vidyasagar University Ethical Clearance (clearance no. IAEC/Revised Proposal/SS01/2016 dt.01.05.2016). Five groups were assigned for the present study with 6 animals each. The animals were conserved in standard cages. They were provided with food pellets and water *ad libitum*. Treatment for each group was done according to Table 1. Blood was collected after 4 h of administration. Administration was done intravenously. There were no visual behavioral changes in the animals after treatment.

2.4. Plant material

The dried bark of *Alstonia scholaris* Linn was obtained Department of Botany, Vidyasagar University(Voucher specimen**(AS-SS101).** 10 g powdered *Alstonia scholaris* Linn bark sample and 80 % methanol as the extraction solvent was used.

2.5. Silver nanoparticle synthesis with Alstonia scholaris Linn bark extract (AgNP-AS)

The dried methanolic plant material was weighed and dissolved in sterile distilled water(200 mg/mL). The concentration of plant material to metal ion were varied (10:1, 20:1, 40:1 & 60:1). 2 mM concentration of $AgNO_3$ was optimal for the preparation. $AgNO_3$ at a concentration of 2 mM was prepared. The synthesis of silver nanoparticle was done by mixing plant extract (10 mL) with 1.2 mL of 2 mM AgNO3 and 18.8 mL distilled water in the volume of 30 mL and heated at 80▫C and stirred at 550 rpm(**Tarson:Digital Spinot**) until the colour of the solution changed from brown to reddish brown. The cooled solution was centrifuged at 5000 rpm for 10 min.. The collected pellets were used in this study [8]. The life AgNP-AS produced could be used for 2 months.

2.5.1. Optimization of parameters for synthesis of AgNP-AS

Optimization of external factors is critical to control reaction parameters. UV–vis analysis was done to obtain optimum wavelength for AgNP-AS.

2.5.2. Optimization of pH for formation of AgNP-AS

pH was varied from 5 to 9 with a difference of 1 to estimate the optimal pH of AgNP-AS.

2.5.3. Effect of concentration of $AgNO_3$

0.5 −3 mM concentration of AgNO3 were studied. Concentration of $AgNO_3$ for synthesis of AgNP was selected by UV–vis absorption spectroscopy.

2.5.4. Optimum temperature for the formation of AgNP-AS

The temperature was varied from 20 to 80 °C with a difference of 10 °C to see the effect on the formation of AgNP-AS.

2.6. Characterization of AgNP-AS

2.6.1. UV–vis analysis

The colour change of AgNPs of *Alstonia scholaris* Linn was) studied by Shimadzu 1800 (Shimadzu Corporation, Kyoto, Japan) and the spectra was taken after 24 h after adding $AgNO_3$ (320 nm -

2.6.2. FTIR analysis

FT-IR stidies of AgNP-AS was done with FT-IR spectrometer (Perkin-Elmer Spectrum Two) at Central Instrumentation Facility; IIT Kharagpur using the dried powders between 4000–700 cm^{-1} by means of KBr pellet method.

2.6.3. Transmission electron microscopy (TEM)

The size of the nanoparticles were calculated by transmission electron microscopy (JEM 2100 JEOL JAPAN instrument) at Central Instrumentation Facility; IIT Kharagpur. AgNPs was loaded on TEM grids and kept in desiccators and transferred onto a specimen holder. The particle size of AgNP-AS was estimated using Image J 1.45 s software(NIH, USA).

2.6.4. ***X*-ray diffraction study(XRD)**

AgNP-AS was characterized by XRD("X"Pert PRO PANalytical, Netherlands).

2.6.5. DLS and zeta potential activity

AgNP-AS was dispersed in deionised water. It was centrifuged for 15 min at 25 °C with 5000 rpm and the supernatant was collected. The particle distribution was examined in ZETA sizer Nanoseries,Malvern instrument Nano Zs 90.

2.6.6. Scanning electron microscopy and EDAX

AgNP-AS was allowed to dry in vaccum.. Morphological analysis was done by ZEISS EVO 60;ESEM, Model 8113(Made in England). Elemental analysis was performed using EDX in SEM(Oxford INCA Penta FETX3).

2.7. Lethality

LD_{50} of viper venom was estimated by the method of Theakston & Reid(1981) [9].

(A) (B)

(C) (D) (E)

Fig. 1. Synthesis of silver nanoparticle based *Alstonia scholaris* Linn bark extract(AgNP-AS).
(A) Bark of *Alstonia scholaris* Linn;(B) Synthesis of AgNP-AS; (C) 2 mM Silver nitrate solution (D) Colour development of AgNP-AS; (E) Aqueous extract of *Alstonia scholaris* Linn.

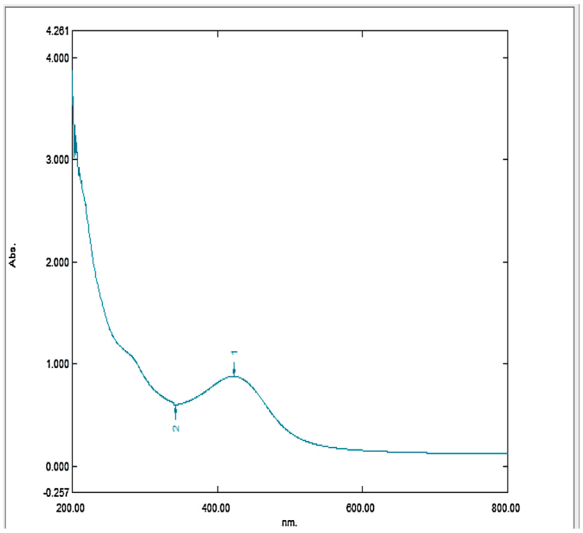


Fig. 2. UV–vis spectra of silver nanoparticle based *Alstonia scholaris* Linn bark extract(AgNP-AS).

2.8. Biochemical estimation of serum proinflammatory markers

For the biochemical estimation of serum proinflammatory markers 6 groups were taken (Table 1). Blood collected from swiss albino mice by retro-orbital plexus. It was clotted for 30 min at 37 °C. Colorimetric determination was done following the method of Serum LDH [10], Serum IL6(Hirano, 1998) [11] and TNFα (Kwon et al; 1993) [12]were estimated by ELISA reader with R& D kit from Thermofisher Scientific (USA) as per manufacturer's instruction.

2.9. Statistical analysis

Results were expressed as Mean ± SD. Results were analyzed using one way ANOVA. Differences were considered as statistically significant at $P < 0.05$ are compared to control(s).

3. Results

Yield from the plant extract was 4%. AgNP-AS was dark brown (Fig. 1) in colour and was stable for 60 days at 8 ± 2 °C. The optimum concentration of *Alstonia scholaris* Linn for nanoparticle synthesis was

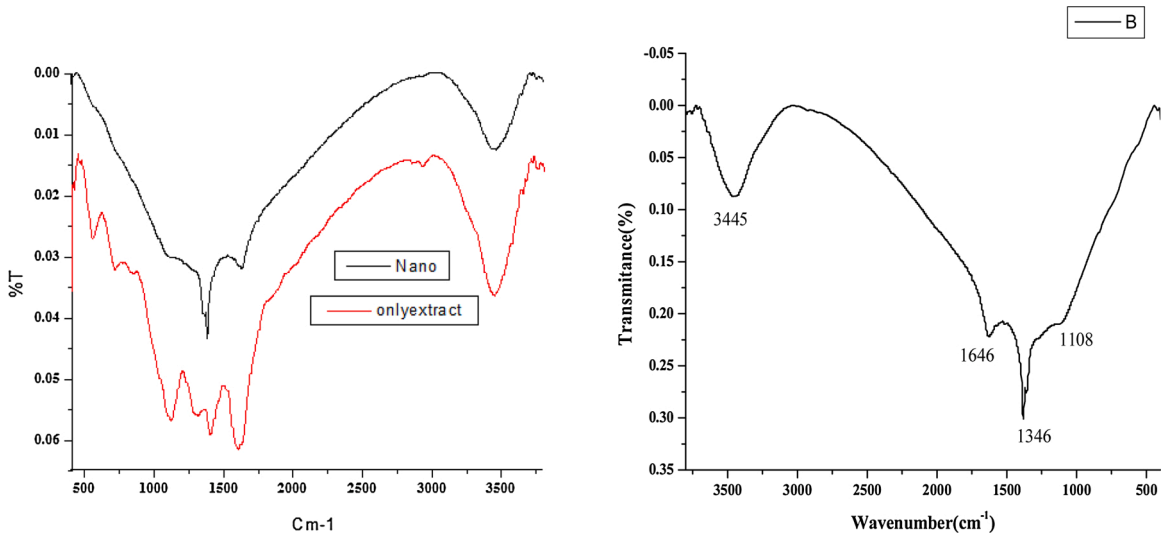


Fig. 3. FT-IR analysis of silver nanoparticle based *Alstonia scholaris* Linn bark extract(AgNP-AS).
(A) FT-IR of *Alstonia scholaris* Linn crude extract(AS) and AgNP-AS ;(B) FT-IR of AgNP-AS.

10gm/l and optimal temperature was 40 °C. It was observed that acidic pH(3–5) was inhibiting synthesis for AgNP. pH 9 was optimum for synthesis of AgNP-AS. In earlier studies it was established that alkaline pH is favourable for synthesis of AgNP [13].

3.1. UV–vis analysis

UV–vis spectra of AgNP-AS showed localized Plasmon resonance of AgNP (Fig. 2). AgNP-AS turned dark brown on addition of $AgNO_3$. The overall reaction indicate the bioreduction of Ag + to Ag^{0}.The maximum absorbance peak was found at 432 nm [14]·

3.2. FT-IR analysis

FT-IR spectra shows peaks at 3445 cm-1 representing a hydroxyl (−OH) group. The peaks at 1646 cm-1 represents double bond(C═C). The peaks 1346 cm-1 and 1108 cm-1 show amine (C—N) and carbonyl (C═O) of proteins, (Fig. 3) [15].

3.3. Transmission electron microscopic studies

Transmission electron microscopy shows measured average particle size for AgNP-AS of 14 nm (Fig. 4). Different capping agents in the extracts has been reported earlier [16].

3.4. X-ray diffraction studies

X-ray diffraction pattern of AgNP-AS had Bragg's reflections of (111), (200), (220) and (311) planes, which confirms the face-centered cubic (FCC) crystalline structure of silver (Fig. 5) [17].

3.5. Dynamic light scattering

The hydrodynamic diameter of AgNP-AS was found to be 115.87 nm (Fig. 6A & B). The zeta potential of AgNP-AS was (-29.8 mV).Several earlier studies validate the study [18,19].

3.6. SEM and EDAX analysis

Fig. 7(A–D) illustrates the SEM studies of AgNP-AS The histogram of AgNP-AS ranges from 20 to 50 nm.The EDAX confirmed a single peak for silver indicating that the silver was pure. The strong signal for silver indicate the crystalline property of silver. Peak at 3KeV was detected by surface plasmon resonance Fig. 7(E & F).

3.7. Neutralization studies of Viper russelli venom(VRV) with AgNP-AS

The LD50 of venom was found to be 2 µg/ 20gm mice. In the neutralization studies with viper venom, *Alstonia scholaris* extract(AAS) (GroupIII) gave 74 % protection against viper venom mediated rise in serum LDH levels, compared to AgNP –AS which gave 80 % protection in Group IV. Antivenom(AVS) gave upto 83 % in Group V.

In the neutralization studies of the pro-inflammatory markers, AgNP-AS gave 62 % protection against venom mediated rise in serum IL6 in Group II after 4 h of venom treatment. AAS gave 52 % protection in Group III and antivenom gave 79 % protection in Group V.

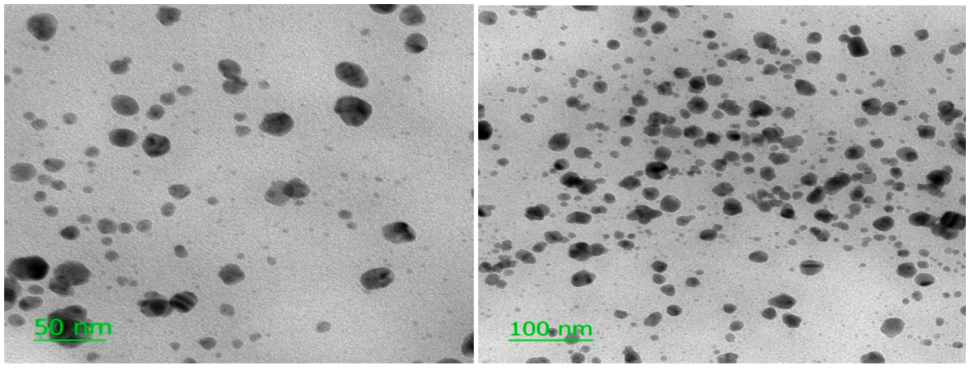
AgNP-AS gave significant protection(52 %**)** against venom mediated rise in TNFα in Group IV followed by AVS(89 %) in Group V. AAS gave 31 % protection in Group III (Table 2).

4. Discussion

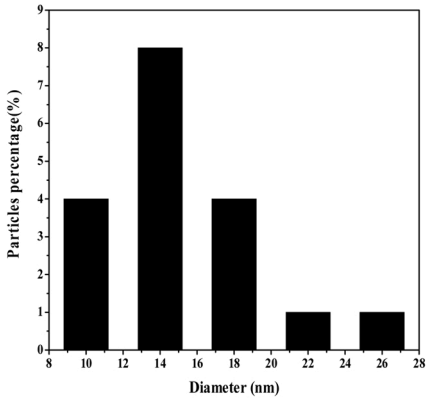
Nanoparticle production has been used for medicine industry [20]. There are several methods of nanoparticle synthesis like chemical diminution of metallic ions in aqueous solutions with or without stabilizing agents [21] thermal disintegration in organic solvents [22], photoreduction in reverse micelles [23,24] and radiation chemical reduction [25,26] have been reported in the literature.

In the present study the colour of AgNP-AS was changed into dark yellowish-brown colourized solution, which indicated that the formation of silver nanoparticles [27,28].The Bragg's reflections for AgNP-AS were(111), (200), (220) and (311) planes, which confirms the face-centered cubic (FCC) crystalline structure of silver [29,30]··Transmission electron microscopy showed measured average particle size for AgNP-AS of 14 nm. The particle size of AgNP-AS ranged from 14−20 nm. Earlier in a similar study, silver nanowires (AgNW) decorated with silver nanoparticles (AgNP) mediated *Mangifera indica* leaf extract was investigated which showed excellent antibacterial activity against *E.coli*. [31] In another study bimetallic Ag/Cu nanoparticles mediated green synthesis using *Opuntia ficus-indica* plant extract [32].

Antisnake venom herbal compounds conjugated with nanoparticles have immense potential as antidote against snake venom action. In an earlier study, curcumin was conjugated with gold nanoparticle neutralized viper venom in animals models [33]. In another study; Saha et al. (2015) investigated the venom neutralization potential of *Vitex negundo* Linn in experimental animal models [34].



(A)



(B)

Fig. 4. A) Transmission Electron microscopic image of AgNP-AS; B) Particle size distribution from TEM.

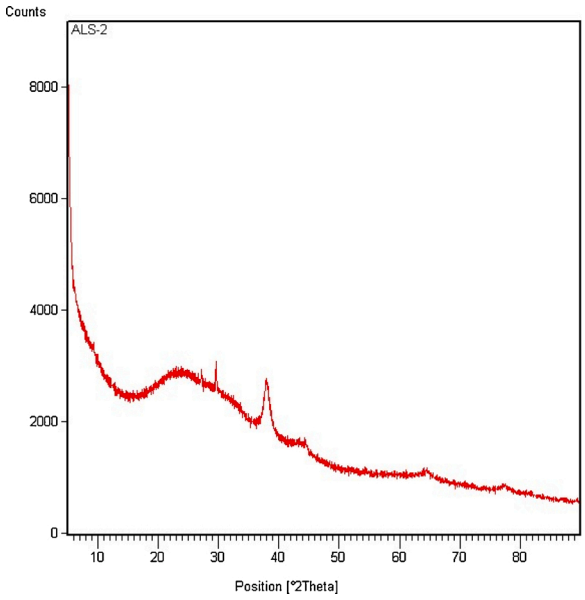


Fig. 5. X-ray diffraction of silver nanoparticle based *Alstonia scholaris* Linn bark extract(AgNP-AS).

Viper venom mediated myotoxic(LDH) and pro-inflammatory markers(IL6, TNFα) were studied. Viper venom attributed primarily to the phospholipase A_2s, metalloproteinases and L-amino acid oxidases contained in their venom. The inflammatory response of snake venoms has been associated with a marked increase of the cytokines IL-1β, IL-6, IL-8, IL-10 and TNF-α.

TNF-α mediates immune response to infections and cancer and in inflammation [35,36].IL-6 is produced by cell types during infection, trauma, and immunological challenge. In the current study, bio-synthesized AgNP-AS significantly neutralized viper venom induced myotoxicity and proinflammatory markers. In a earlier study, the effect of silver nanoparticles on antioxidant and pro-oxidant balance in murine models was investigated using total antioxidant capacity (TAC), thio-barbituric reactive species (TBARS),protein carbonyl (PROTC) levels, reduced glutathione (GSH) levels and catalase (CAT) activity [37]. In a similar study, AgNP-induced hepatotoxic effects of *Clarias gariepinus* was investigated in a dose –dependent manner [38]. The study has potential for investigation of future drug development in the field of snake bite management and overcome the drawbacks of the antivenom therapy. However further studies are warranted to investigate the potential of this nanoparticle based herbal in snake venom induced pathophysiology and its mechanism.

5. Conclusion

Development of future anti-microbial herbal medicine with improved potential and less toxicity is a desired area in drug discovery. However further characterization of bioactive ingredients of the plant bark and efficacy of the plant material should be investigated. Our findings provides insights into new antivenom agents with synergistic enhancement of mechanism. The present study has a wide application from the perspective of its use as a substitute for traditional antivenom therapy. Besides the reported plant is widely available and herbal medicine has lesser side-effects as compared to antivenom therapy.

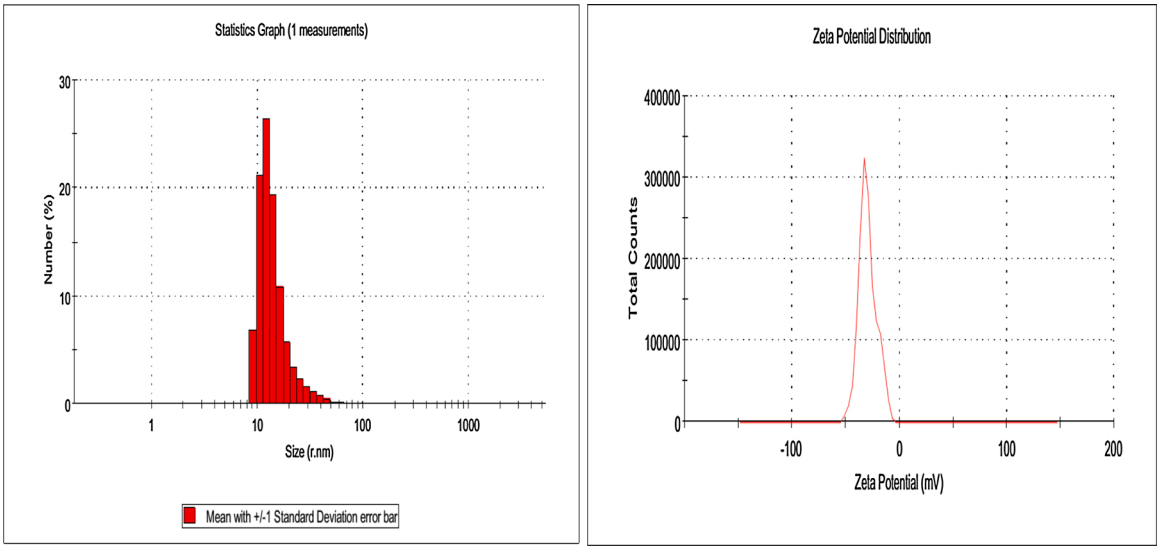
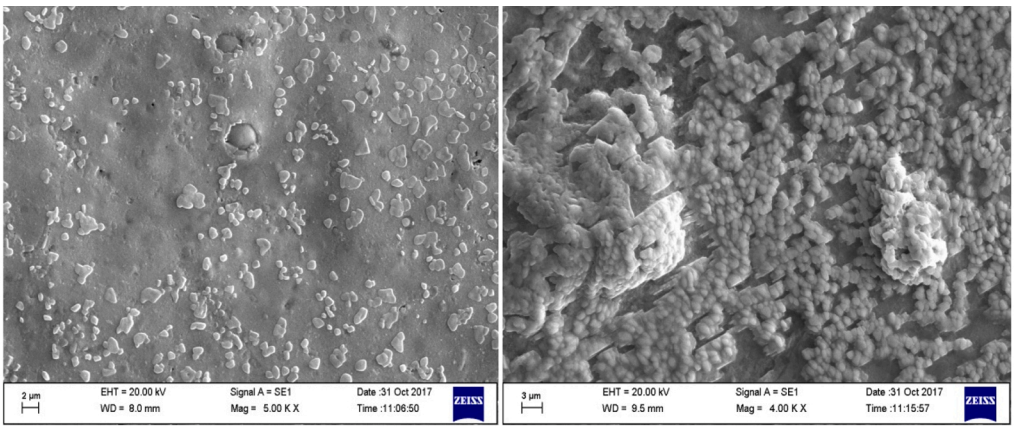
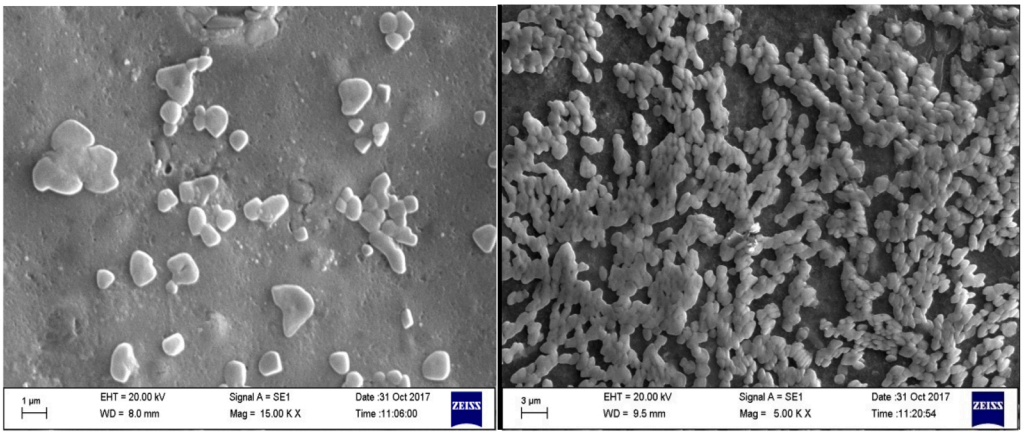


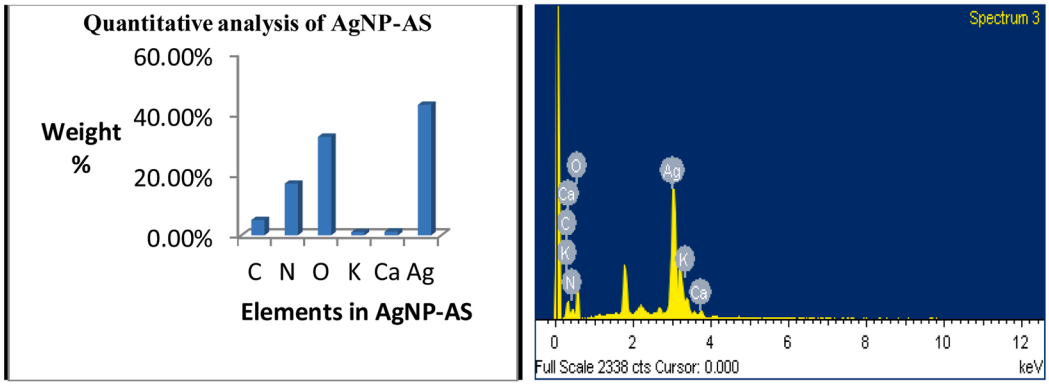
Fig. 6. (A): Dynamic Light scattering of hydrodynamic diameter (115.87 nm) and Fig. 6(B) zeta potential(-29.8 mV)of AgNP-AS.



(A) (B)



(C) (D)



(E) (F)

Fig. 7. SEM pictures of silver powder granulates deposited on carbon strip(A-D);EDAX analysis of AgNP-AS(E &F).

Table 2
Neutralization of viper venom mediated rise of serum myotoxicity and proinflammatory markers by AAS and AgNP-AS.

Groups(n = 6)	LDH(U/L)	IL6(pg/mL)	TNFα(pg/mL)
Group I	135 ± 0.05	31.5 ± 0.01	17.2 ± 0.05
Group II	603 ± 0.01	303 ± 0.05	225 ± 0.01
Group III	155 ± 0.02*	145 ± 0.01*	155 ± 0.01*
Group IV	120 ± 0.14*	115 ± 0.14*	109 ± 0.01*
Group V	105 ± 0.02*	65 ± 0.02*	25 ± 0.15*

AAS and AgNP-AS on.
* $P < 0.05$ as compared to Group II.

CRediT authorship contribution statement

Kanchan Saha: Conceptualization. **Sumana Sarkhel:** Data curation. **Upasana Chatterjee:** Formal analysis. **Sumana Sarkhel:** Investigation. **Poulomi Parua:** Data analysis. **Rituparna Ghosh:** Conceptualization, **Koushik Mana:** Investigation.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgement

The study was undertaken from the funds of Department of Science and Technology (Ref:607(Sanc)/ST/P/S&T/5G-3/2016 dated 17/10/2016).

References

[1] P.K. Mukherjee, P. Venkatesh, M. Venkatesh, S. Ponnushankar, K. Yaseen, Strategies of revitalization of traditional medicine, Chin. Herbal Med. 2 (11) (2010) 1–15, https://doi.org/10.3969/j.issn.1674-6384.2010.01.001.
[2] V.K. Sharma, R.A. Yngard, Y. Lin, Silver nanoparticles: green synthesis and their antimicrobial activities, Adv. Colloid Interface Sci. 145 (2009) 83–96, https://doi.org/10.1016/j.cis.2008.09.002.
[3] C. Krishnaraj, E.G. Jagan, R. Ramachandran, S.M. Abirami, N. Mohan, P. T. Kalaichelvan, Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. Plant growth metabolism, Process. Biochem. 47 (2012) 651–6581, https://doi.org/10.1016/j.procbio.2012.01.006.
[4] K.S. Mukunthan, E.K. Elumalai, N.P. Trupti, V. Ramachandra Murty, *Catharanthus roseus*: a natural source for the synthesis of silver nanoparticles, Asian Pac. J. Trop. Biomed. (2011) 270–274, https://doi.org/10.1016/s2221-1691(11)60041-5.
[5] R. Arunachalam, S. Dhanasingha, B. Kalimuthua, M. Uthirappana, B. Rosea, A. B. Mandal, Photosynthesise of silver nanoparticles using *Coccinia grandis* leaf extract and its application in the photocatalytic degradation, Colloids Surf. B Biointerfaces 94 (2012) 226–230, https://doi.org/10.1016/j.colsurfb.2012.01.040.
[6] P.J. Houghton, I.M. Osibogun, Flowering plants used against snakebite, J. Ethnopharmacol. 39 (1993) 1–29, https://doi.org/10.1016/0378-8741(93)90047-9.
[7] M.R. Khan, A.D. Omoloso, M. Kihara, Fitoterapia 74 (2003) 736–740, https://doi.org/10.1016/s0367-326x(03)00192-8.
[8] A. Ahmad, P. Mukherjee, S. Senapati, D. Mandal, M.I. Kham, R. Kumar, M. Sastry, Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*, Colloids Surf. B Biointerfaces 28 (2003) 313–318, https://doi.org/10.1016/s0927-7765(02)00174-1.
[9] Reid Theakston, Development of simle standard assay procedures for the characterization of snake venom, Bull. World Health Organ. 61 (6) (1983) 949–956.
[10] Recommendations for the measurement of LDH in Human serum at 30° C, Ann. Bio. Chem. 40 (1982) 87, https://doi.org/10.1016/0009-8981(79)90036-6.
[11] A. Hirano, H. Yano, S. Akita, Therapeutic choice for craniofacial venoms malformation, J. Craniofac. Surg. 17 (4) (2006) 729–735, https://doi.org/10.1097/00001665-200607000-00025.
[12] B. Kwon, K.Y. Yu, J. Ni, G.L. Yu, I.K. Jang, Y.J. Kim, L. Xing, D. Liu, S.X. Wang, B. S. Kwon, Identification of a novel activation-inducible protein of the tumor necrosis factor receptor superfamily and its ligand, J. Biol. Chem. 274 (1999) 6056 61, https://doi.org/10.1074/jbc.274.10.6056.
[13] C. Krishnaraj, E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan, N. Mohan, Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens, Colloids Surf. B Biointerfaces 76 (2010) 50–56, https://doi.org/10.1016/j.colsurfb.2009.10.008.
[14] S. K.Ghosh, S. Kundu, M. Mandal, S. Nath, T. Pal, Studies on the evolution of silver nanoparticles in micelle by UV-photo activation, J. Nanopart. Res. 5 (2003) 577–587, https://doi.org/10.1023/b:nano.0000006100.25744.fa.
[15] P. Shetty, N. Supraja, M. Garud, Synthesis, characterization and antimicrobial activity of Alstonia scholaris bark-extract-mediated silver nanoparticles, J. Nanostruct. Chem. 4 (4) (2014) 161–170, https://doi.org/10.1007/s40097-014-0132-z.
[16] J.Y. Song, B.S. Kim, Rapid biological synthesis of silver nanoparticles using plant leaf extract, Bioprocess Biosyst. Eng. 32 (2009) 79–84, https://doi.org/10.1007/s00449-008-0224-6.
[17] R.M. Ammal, G.V. Bai, Green synthesis of silver nanostructures against human cancer cell lines and certain pathogens, Int. J. Pharm. Chem. Biol. Sci. 4 (1) (2014) 101–111, https://doi.org/10.22376/ijpbs/lpr.2021.11.1.l148-154.
[18] M. Vanaja, K. Paulkumar, G. Gnanajobitha, S. Rajeshkumar, C. Malarkodi, G. Annadurai, Herbal plant synthesis of antibacterial silver nanoparticles by *Solanum trilobatum* and its characterization, Int. J. Met. (2014) (2014) 8, https://doi.org/10.1155/2014/692461.
[19] L.M. Liz-Marzan, I. Lado-Tourino, Reduction and stabilization of silver nanoparticles in ethanolbynonionicsurfactants, Langmuir 12 (1996) 3585–3589, https://doi.org/10.1021/la951501e.
[20] K. Esumi, T. Tano, K. Torigoe, K. Meguro, Preparation and characterization of biometallic Pd-Cu colloids by thermal decomposition of their acetate compounds in organic solvents, J. Chem. Mater. 2 (1990) 564–567, https://doi.org/10.1002/chin.199052020.
[21] M.P. Pileni, Fabrication and physical properties of self-organized silver nanocrystals, Pure Appl. Chem. 72 (2000) 53–65, https://doi.org/10.1351/pac200072010053.
[22] Y.P. Sun, P. Atorngitjawat, M.J. Meziani, Preparation of silver nanoparticles via rapid expansion of water in carbon dioxide microemulsion into reductant solution, Langmuir 17 (2001) 5707–5710, https://doi.org/10.1021/la0103057.
[23] A. Henglein, Physicochemical properties of small metal particles in solution: microelectrode' reactions, chemisorption, composite metal particles, and the atom-to-metal transition, J. Phys. Chem. B 97 (1993) 5457–5471, https://doi.org/10.1021/j100123a004.
[24] A. Henglein, Reduction of Ag (CN)-2 on silver and platinum colloidal nanoparticles, Langmuir 17 (2001) 2329–2333, https://doi.org/10.1021/la001081f.
[25] S.S. Shankar, A. Ahmed, B. Akkamwar, M. Sastry, A. Rai, A. Singh, Biological synthesis of triangular gold nanoprism, Nature 3 (2004) 482, https://doi.org/10.1038/nmat1152.
[26] J.S. Kim, E. Kuk, K.N. Yu, J.H. Kim, S.J. Park, H.J. Lee, Nanomed. Nanotechnol. Biol. Med. 3 (2007) 95–101, https://doi.org/10.1016/j.nano.2014.04.007.
[27] P. Gong, H.M. Li, X.X. He, K.M. Wang, J.B. Hu, W.H. Tan, Preparation and antibacterial activity of Fe_3O_4Ag nanoparticles, Nanotechnology 18 (2007) 285–604, https://doi.org/10.1088/0957-4484/18/28/285604.
[28] H. Xu, M. Kall, Surface-plasmon-enhanced optical forces in silver nanoaggregates, Phys. Rev. Lett. 89 (2002) 246–802, https://doi.org/10.1103/physrevlett.89.246802.
[29] V. Kumar, S.K. Yadav, Plant-mediated synthesis of silver and gold nanoparticles and their applications, J. Chem. Technol. Biotechnol. 84 (2009) 151–157, https://doi.org/10.1002/jctb.2023.
[30] S. Poopathi, L.J.D. Britto, V. Li Prab, C. Mani, M. Praveen, Synthesis of silver nanoparticles from *Azadirachta indica*—a most effective method for mosquito control, Environ. Sci. Pollut. Res. 22 (2015) 2956–2963, https://doi.org/10.1007/s11356-014-3560-x.
[31] S. Horta-Piñeres, R. Britto Hurtado, D. Avila-Padilla, M. Cortez-Valadez, N. S. Flores-López, M. Flores-Acosta, Appl. Phys. A 126 (2020) 15.
[32] J.-G. Bocarando-Chacon, M. Cortez-Valadez, D. Vargas-Vazquez, F. Rodríguez Melgarejo, M. Flores-Acosta, P.G. Mani-Gonzalez, E. Leon-Sarabia, A. Navarro-Badilla, R. Ramírez-Bon, Phys. E 59 (2014) 15–18.
[33] S. Ghosh, A. Gomes, Russelli's viper(Daboia russelli russelli) neutralising efficacy of curcumin gold-nanoparticle(C-GNP) in experimental animal models, J. Toxins 3 (2016) 6.
[34] K. Saha, S. Ghosh, S. Ghosh, S.C. Dasgupta, A. Gomes, A. Gomes, Neutralization of Naja kaouthia venom induced acute toxicity and stress response with herbal gold nanoparticles(VN-GNP) in experimental animal models, J. Toxins 2 (2015) 8.
[35] M.M. Magalhães, M.E.S. Pereira, C.F.S. Amaral, et al., Serum levels of cytokines in patients envenomed by Tityus serrulatus scorpion sting, Toxicon 37 (8) (1999) 1155–1164, https://doi.org/10.1016/s0041-0101(98)00251-7.
[36] M. Howard, T. Muchamuel, S. Andrade, S. Menon, Interleukin 10 protects mice from lethal endotoxemia, J. Exp. Med. 177 (4) (1993) 1205–1208, https://doi.org/10.1084/jem.177.4.1205.
[37] A.O. Docea, D. Calina, A.M. Buga, et al., The effect of silver nanoparticles on antioxidant/pro-oxidant balance in a murine model, Int. J. Mol. Sci. 21 (4) (2020) 1233, https://doi.org/10.3390/ijms21041233. Published 2020 Feb 12.
[38] Mervat Naguib, Usama M. Mahmoud, Imam A. Mekkawy, Alaa El-Din H. Sayed, Hepatotoxic effects of silver nanoparticles on *Clarias gariepinus*; Biochemical, histopathological, and histochemical studies, Toxicol. Rep. 7 (2020) 133–141.