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Antivenom potential of chitosan gold nanoparticles

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Nanoparticle molecules have gained interest in medicine due to their properties of increased cellular uptake and efficacy. Keeping this in mind, the current study aimed to explore the neutralizing potential of chitosan gold nanoparticles (CH-AuNP) against the venom of *Vipera russelli*, in *in vitro* and *invivo* studies. UV-Vis Spectra of CH-AuNP exhibited absorbance at 530 nm. Dynamic light scattering report corroborated that there was a formation of monodispersed nanoparticles with hydrodynamic diameter of about 89.65 nm. The zeta potential of CH-AuNP was found to be 24.7 mV. X-ray diffraction analysis of CH-AuNP which confirmed the cubic crystal shape of the gold nanoparticles. SEM studies of the synthesized CH-AuNP exhibited particle sizes ranging from 7 to 18 nm.*In vitro* haemagglutination, enzyme assays and coagulation effect of venom on blood were tested with and without the presence of CH-AuNP. *Invivo*assays included lethality, haemorrhage and nephrotoxicity with and without the presence of CH-AuNP. Results obtained in the anti-haemolytic assay with chitosan and CH-AuNP revealed 86.72% and 93.01% protection against viper venom induced haemolysis. The CH-AuNp also accorded significant protection against venom induced coagulation and proteolytic activity. The *invivo* studies revealed that the CH-AuNP neutralized venom induced lethargy and haemorrhagic activity. It can therefore be stated that the CH-AuNP can potentially have a therapeutic effect on venom induced patho-physiological changes.

Keywords: Russell's viper venom, Chitosan gold nanoparticle, Lethality, Haemorrhagic action,Nephrotoxicity

Snakebite envenoming has long been considereda medical menace and is globally associated with mortality and dysfunction[1].Antivenoms are the most commonly used treatment method butefficacy testing needs *in-vitro* studies in conjunction with *in-vivo* animal toxicity tests to examine the potency of antivenoms and assess the counteracting dimensions of the same. In *invivo* studies, animal models specifically mice modelshave long been used to evaluate venom induced lethality and for studying the counteracting action of antivenoms[2].In India, the Russell's viper (*Vipera russelli*) is one of the most commonly found venomous snakes. The venom primarily acts *via* haemorrhagic action. The haemorrhagic action by *Viperid* venoms have mostly been ascribed to the activity of snake venom metalloproteinases.Research into the pathophysiology of snake envenomation has long been advocated for a thorough assessment of the antagonistic properties of antivenom since it has been considered a benchmark of efficacy.Research has also indicated the need for further studies on the inhibiting potential of antivenom on haemorrhagic action, defibrinogenating, myotoxicity and dermonecrotic activities all of which occur during envenomation by varied snakes[3]. Chitin is a polymer formed by the polymerization of the residues of N-acetylglucosamine and glucosamine through theβ-1,4-glycosidic bonds.Chitin and chitosan are common components of fungal cell walls[4]. Chitosan has also been found to be favourable for inhibiting Gram-negative bacterium in comparison to Gram-positive bacterium due to the negative chargeon thecell surface enabling more interaction with the positively charged chitosan[5].Past research has extensively studied the anti-inflammatory and pro-inflammatory attributes of chitosan and its byproducts[6]. Some studies also focused on the antitumor potential of low molecular weight chitosan[7].A past study reported that the hyaluronidase in *Naja naja* venom was inhibited by chitosan[8]. Keeping this in mind, the authors of the current study aimed to investigate the potential of chitosan and chitosan gold nanoparticles to inhibit the toxicity of Russell's Viper venom. To achieve the objective, *invitro* and *invivo* studies were conducted. The *invivo* studies involved the use of mice models to achieve

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the desired objectives of inhibiting lethality, haemorrhagic activity and nephrotoxicity.

The *invitro* studies involved studying the effect of chitosan and CH-AuNPfor inhibiting haemolysis, procoagulant activityand proteolytic activity of the *Vipera russelli*venom.

Materials and Methods

Venom

Commercial freeze-dried venom powder of the Russell's viper was purchased from the Irula Snake Catcher Cooperative Society, Kancheepuram, Chennai and stored at 4^{o}C until further use. For use in studies, thefreeze-driedvenom powder was reconstituted (mg/mL, w/v) in 0.9 % (w/v) saline andcentrifuged at 3000 rpm for 20 min. Following this the supernatant was collected for further use.

Chemicals

Gold chloride($HAuCl_4$) was procuredfrom Sigma-Aldrich Chemicals, USA.Chitosan was obtainedfrom marine shrimp shells(deacetylated in1% solution of acetic acid;Merck, India). The degree of deacetylation was set at 75% and the resultant solution was stored at 4^{o}C until further use.

Animals

Swiss albino mice aging 6-8 weeks old(20±2g in weight) were obtained from a local animal supplier.The mice were maintained in polypropylene cages and provided with food and water *ad libitum*.Animals were maintained as per the standard guidelines of CPCSEA (clearance no.VU/IAEC/ CPCSEA/8/6/2022).

Acute toxicity of chitosan

Acute toxicity study was carried out in healthy Swiss albino mice (20±2 g in weight) using the 'Upand Down' method of testing in mice in accordance with the Organisation for Economic Co-operation and Development (OECD 2008)[9].Animals were randomly allocated into two groups of 6 mice each and kept 3h fasting prior to chitosan administration. Group-I served as the control and the mice were intraperitonially administered with 500µL 0.9 % *w/v*NaCl. Group-II served as treated group. Each animal was observed after dosing for the first 5 min for signs of regurgitation and kept in a cage. Each was then observed every 15 min in the first 4 h after dosing, every 30 min for 6 h and daily for 48 h for behaviouralsigns of toxicity (skin and fur, eyes and mucus membranes, behavior pattern, tremors, salivation, sleep, mortality,ill health or any visible reaction to treatment) according to the specifications of the OECD (2001). The animals were monitored for a total of 14 days for the longterm possible lethal outcome. The body weights of the animals were measured on days 1st, 7th and 14thusing a sensitive balance.

Synthesis of chitosan gold nanoparticle (CH-AuNP)

Chitosan (0.2%*w/v*) was prepared by mixing in 0.5% acetic acid. After that the chitosansolution was stirred to create a homogeneous solution. 2 mL of 1.25 mMHAuCl4was added dropbydrop to the chitosan solution. At 80^{o} C, the mixture wasagitated for 4 h. The colourless chitosan solution became violet indicating that AuNPswere synthesized.The solution was heated to 80°C and agitated at 500rpm using a magnetic stirrer.(Fig. 1).The solution was observed for colour changes and the formation of a ruby red colour indicated the formulation of CH-AuNP.

Optimization of chitosan gold nanoparticle synthesis (CH-AuNP)

In this part of synthesis of nanoparticles, $HAuCl_4$ initial concentration effect was studied. $HAuCl_4$was mixed with millipore water at different concentrations 0.5-4 mM and mixed with 26.25mL of 0.2% w/v chitosan[10]. pH was varied from 4 to 10 with a difference of 1 to estimate the optimal pH of CH-AuNP. The temperature was varied from 20 to 90^{o}C with a difference of 10^{o}C to see the effect on the formation of CH-AuNP.

Characterization of synthesized CH-AuNP

The primary characteristics of the synthesized CH-AuNP were observed using ultraviolet visible spectroscopy. The synthesized CH-AuNP was stored for 4 h following which the optical density (OD)was observed in the range of 300-700nm using an ultraviolet visible spectrometer (Shimadzu1800, Kyoto, Japan). A graph was plotted based on the OD measurements using the automated UV probe software. FTIR spectroscopy of CH-AuNP was performed at Central Research Facility Laboratory, Indian Institute of Technology Kharagpur. TheCH-AuNP was pelletedwith potassium bromide (KBr) at a 1:10 ratio and the FTIR spectroscopic measurements were carried out from 4,000 to 700 cm^{-1}. The hydrodynamic diameter particle (Z-average) and zeta potential distribution of the CH-AuNPs were characterized via the dynamic light scattering technique by using a zeta size analyserat the Indian

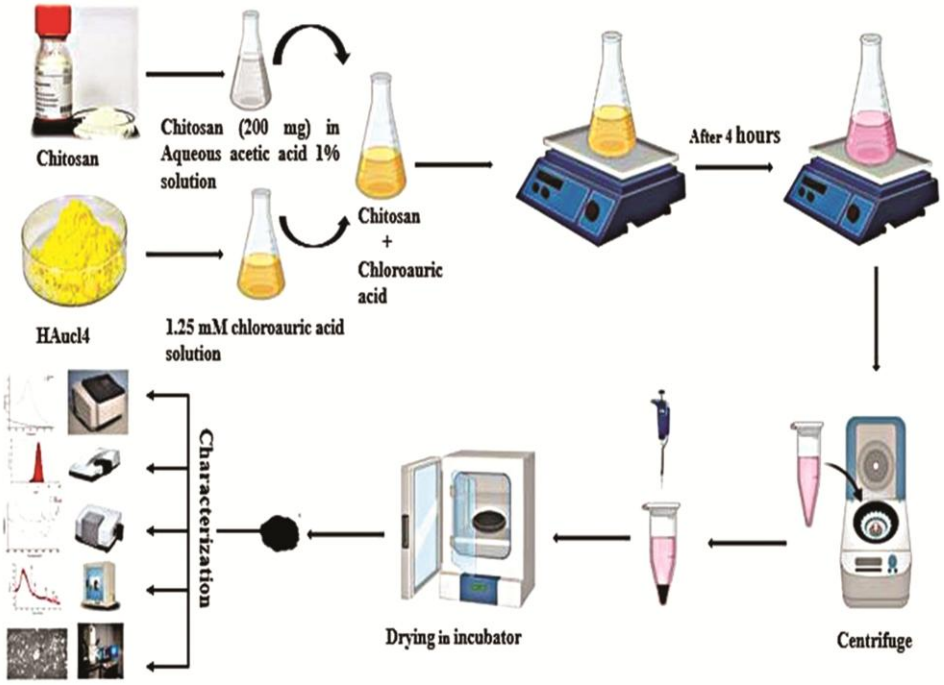

Fig. 1 — Illustrated methodology for the synthesis and physical characterization of chitosangold nanopaticle.

Institute of Chemical Biology, Kolkata.The analysis of elemental studies was evaluated using EDX in SEM (Oxford INCA Penta FETX3). Energy-dispersive analysis of X-ray spectroscopy(EDAX) was also conducted to perform the elemental analysis of gold.

Invitro inhibition of Vipera russelli venom

Venom-induced direct haemolytic assay and neutralization studies

Direct haemolytic assay induced by *Vipera russelli* venom and subsequent neutralization studies were carried out *in vitro* by using the Red blood cell (RBC) assayas described by Vineetha *et al.*[11]. For the purpose of this study, goat blood was collected from a local slaughterhouse and ethylenediaminetetraacetic acid (EDTA) was added to prevent coagulation. Following this, 5 mL of whole blood was gently centrifuged at 900 rpm for 10 min. The clear supernatant was discarded following centrifugation and the resultant pellet was washed thrice with physiological saline solution (0.9% w/v) and reconstituted in the same. Next, 0.5 mL of reconstituted RBC was mixed with 5 mL phosphate buffer solution (pH 7.4) mixture and used as control. For 100% haemolysis 0.5 mL of reconstituted RBC was mixed with 5 mL of double distilled water. For the test samples 5mL of VRV /Chitosan/CH-AuNP was mixed with 0.5mL of reconstituted RBC. All the sample aliquots were incubated at 37°C for 1h and then centrifuged for 20 min at 2000 rpm. The resultant supernatants were collected in fresh tubes for measuring the OD at 540nm in an Ultraviolet-visible spectrophotometer. Distilled water was used as blank control. The haemolysis was estimated by the formula.In this study, *Vipera russelli* venom was used to induce haemolysis. Graded concentrations of chitosan/CH-AuNP were mixed with 1µg of venom for neutralization.

$$\frac{Experimental\ \ sample - Control\ \ sample}{100\%\ hemoly\ sis\ (RBC + D.Water\ )} \times 100$$

Procoagulant activity

The procoagulant activity of chitosan/CH-AuNP was evaluated following the method described in an earlier study by Theakston &Reid[12]. The VRVwas dissolved in 100 µL phosphate buffer solution (pH 7.2) before adding citrated mice blood to the solution followed by incubation at 37°C. The coagulation time was calculated by establishing the Minimum Coagulant Dose (MCD) of venom *i.e.*, the least dose of venom which initiated RBC coagulation within 60 second.The Minimum Coagulant Dose (MCD) of *Vipera russelli* venom was to be found 20µg. To evaluate the procoagulant activity, chitosan/CH-AuNP was incubated for 30 min along with the MCD of venom. A mixture of 0.1 mL of phosphate buffer along with 0.3 mL of citrated blood was used as control and the clotting time was estimated.

Anti-proteolytic activity

Anti-proteolytic potential was investigated by the method described in earlier studies[13,14]. The methodology was modified and employed in the current study to measure the proteolytic activity of *Vipera russelli* venom (VRV). 2 mL of 1% casein suspended in 0.25 M sodium phosphate buffer (pH 7.75) and 0.1 mL of venom (2 µg) in physiologic saline were mixed and incubated for 1 h at 37^0 C. The undigested casein was precipitated and the reaction was terminated by adding 2 mL of 5% trichloroacetic acid. After centrifugation at 10,000 rpm for 10 min, the absorbance of the supernatant was measured at 280 nm in a UV spectrometer. One unit of proteolytic activity was defined as the increase of 0.001 absorbance units at 280 nm per hour. Anti-proteolytic activity of chitosan/CH-AuNP was also evaluated against *Vipera russelli* venom. All experiments were performed in triplicate and the results presented as average values with standard errors.

***In vivo* neutralization of Vipera russelli venom**

Neutralization of lethality

The median lethal dose was determined by injecting different concentration of venom in a constant volume of salinein male Swiss albino mice by intravenous route. Mice were observed for behavioural changes, toxicity and survival time was recorded. Accurate lethal dose was calculated by Meier &Theakston method[12].For neutralization study different concentrations venom was pre-incubated with chitosan/CH-AuNP.After injection, deaths were recorded at 24 h and the behavioural changes were recorded.The minimum lethal dose (MLD) of venom was found 2µg in 20 gm of albino mice. In neutralization study,1MLD venom 100% neutralization by chitosan/chitosan gold nanoparticles considered as a 1fold Protection.

Anti-haemorrhagic activity of CH-AuNP

The minimum haemorrhagic dose (MHD) of *Vipera russelli* venom defined as the least amount of venom, which when injected intradermally *(i.d.)* into mice (20±2 g,n=6) results in a haemorrhagic lesion of 10 mm diameter 24 h of exposure[15]**.***In vivo* minimum haemorrhagic dose (MHD) of *Vipera russelli* venomwas found to be 10µg/20 gm body weight of albino mice.For neutralization of haemorrhagic activity,animals were divided into four groups (no of animal=6), Group-Isaline control received 0.9 %*w/v* NaCl solution, Group-II venom treated, Group-III venom/chitosan treated and Group-IV venom/CH-AuNP treated respectively.Venom was incubated with chitosan/CH-AuNPat 37^o C for 30 min, followed by intradermal injection into the test mice.After 24 h, the animals were euthanizedby inhalation of carbon dioxide in a carbon dioxide chamber following the guidelines of the CPCSEA. Digital callipers were used to measure the haemorrhagic spots on the dorsal patch of skin.Minimum haemorrhagic dose (MHD) of venom was found 10µg in 20 gm of albino mice. In neutralization study,1MHD of venom 100% neutralization by chitosan/chitosan gold nanoparticles considered as a 1fold Protection.

Neutralization of nephrotoxicity

Nephrotoxicity was determined according to the method described in earlier studies by estimating the serum biomarkers of uric acid and creatinine[16].The test mice were divided into four groups of 6x mice each and treated intravenously. Group I received saline and GroupII was subjected to venom (half the LD_{50}).GroupIII and GroupIV weretreated with the venom pre-incubated with the chitosan and CH-AuNP for 15 min at 37 °C. After 4 h of exposure, blood was drawn from the orbital plexus of the mice and theserum was collected from the drawn blood. Creatinine and uric acid levelswere estimated using commercially available Tulip biochemical Kit.

Statistical analysis

Results are shown as Mean±SD. Results were analysed using one way ANOVA. The difference was given as statistically significant at $P<0.05$ are compared to venom control.

Results

Characterization of synthesized nanoparticles

The reduction of $HAuCl_4$ was visibly detected by colour change of Chitosan solution from colour less into ruby red colour confirming the formation of gold nanoparticles.UV-vis spectra of the CH-AuNP exhibited an absorbance peak at 530 nm (Fig. 2 & Fig. 3). The results obtained from the dynamic light scattering study indicatedthe formation of monodispersed nanoparticles with a hydrodynamic diameter of approximately 89.65 nm as exhibited by the appearance of a single peak (Fig. 4A). The zeta potential of the gold nanoparticles was observed to be +24.7 mV(Fig. 4B). In FTIR analysis absorption peaks were observed at3440 cm^{-1} which signified the presence of hydroxyl groups. The presence of peaks at 1630 $cm^{-1}$was representative of the C=C stretching

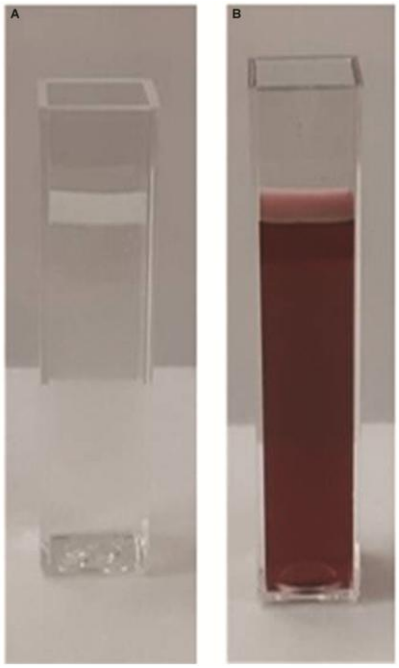

Fig. 2 — Optical analysis of colour alteration in (A) chitosan(B) Synthesizedchitosan gold nanoparticles (CH-AuNP)

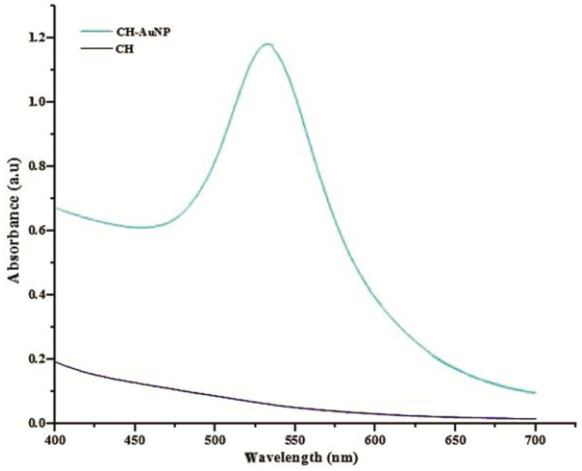

Fig. 3 — UV-Visible spectra of CH-AuNP showing the surface plasmon resonance of nanoparticle

alkenes while the presence of peaks at 1390 cm^{-1} signified the presence of C-H bendingaldehyde. The peaks at 1040 cm^{-1}signified the presence of C-F stretchingfluoro compounds and the peaks at 634 $cm^{-1}$was representative of the C-I stretching halo compound (Fig. 5). In the X-ray diffraction (XRD) analysis of the CH-AuNPfive strong peaks were obtained at 38.2°, 44.4°, 64.7°, 77.7° and 81.9° respectivelywhich corresponded to the planes of (111), (022), (220), (131) and (222) respectively.

This confirmed the cubic crystal shape of gold (Fig. 6). Thescanning electron microscopic studies of

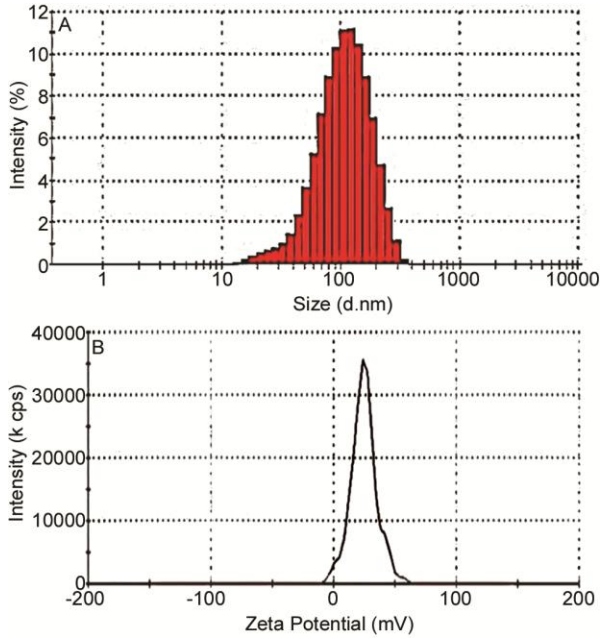

Fig. 4 — (A) Dynamic light scattering (DLS) pattern and (B) zeta potential distribution of chitosan gold nanoparticle (CH-AuNP)

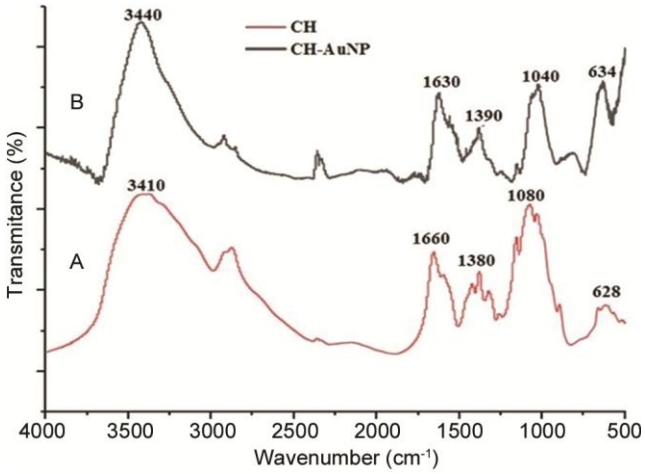

Fig. 5 — FTIR analysis of (A) chitosan (B) chitosangold nanoparticle (CH-AuNP) exhibiting the functional groups as indicated by the presence of peaks.

CH-AuNPexhibited a particle size ranging from 7-18 nm. The energy dispersive X-ray analysis (EDAX) provided elemental analysis and specified the amount of Au (in%) which was denoted by the sharp peaks in the resultant graph (Fig. 7).

In vitro neutralization of *Vipera russelli* venom

Venom-induced direct haemolytic assay

Direct hemolysis of *vipera russelli* venom produced 95.83 % hemolysis and *in vitro* neutralization studies it was observed that the chitosan

when incubated with venom accorded 86.72% protection. Whereaswhen the venom was incubated with CH-AuNP it provided 93.01% protection against venom mediated haemolysis. It is evident from the results obtained for haemolysis studies that chitosan and CH-AuNP shows maximal protection against the venom induced haemolysis at dose of 15mg/mL and 2mg/mL (Table 1) (Fig. 8).

Procoagulant activity

Normal coagulation was obtained by adding blood with PBS and CaCl2. The coagulation period was prolonged by *vipera russellii* venom. In neutralization studies chitosan and CH-AuNP provided significant protection against VRV mediated procoagulant activity at dose of 15 mg/mL and 2 mg/mL (Fig. 9).

Anti-proteolytic activity

It was observed from the results that chitosan significantly neutralized VRV mediatedproteolytic activity.The initial 2 µgproteolytic dose delivered 303 units of proteolytic activity when compared to venom plus PBS as control. It was observed that the chitosan inhibitedthe proteolytic activity of VRV up to 68 % in agraded manner. CH-AuNP was also observed to significantly neutralize the VRV induced proteolytic activitybetween 209 and 61 units with close to 97% inhibition of the proteolytic activity (Table 2).

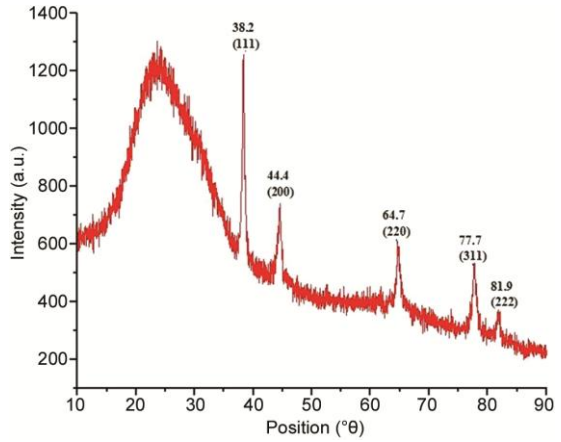

Fig. 6 — X-ray diffraction analysis of chitosan gold nanoparticle (CH-AuNP) exhibiting peaks at (111), (020), (022), (131), and (222), which was indicative of the cubic crystalline structure of gold nanoparticle

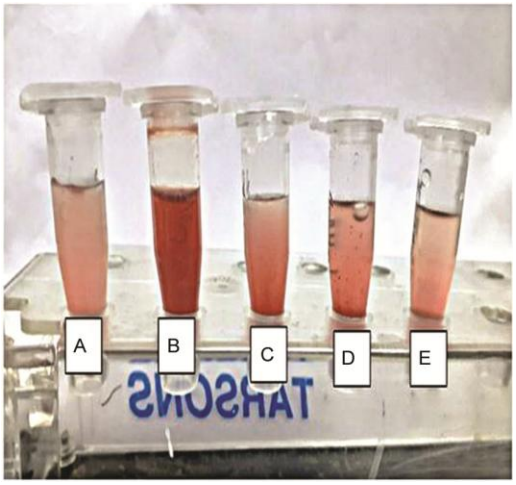

Fig. 8 — Direct haemolytic assay mediated by *Vipera russellii* venom and its neutralization by chitosan and chitosangold nanoparticle (CH-AuNP) in comparison to control

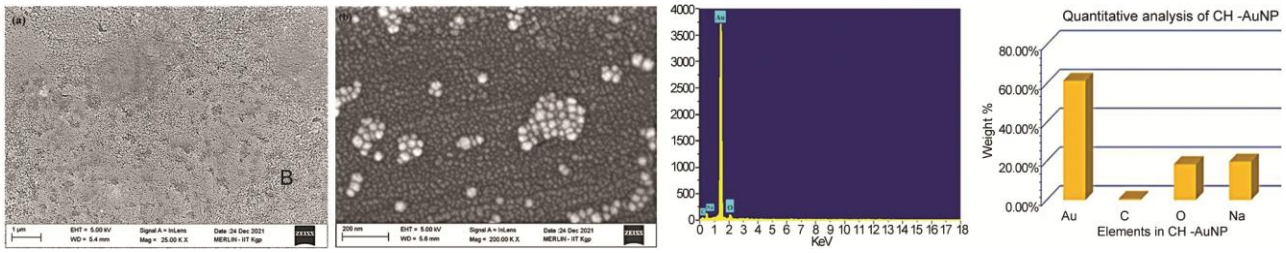

Fig. 7 — SEM images (A &B). Energy dispersive X-ray analysis (EDAX) of chitosan gold nanoparticle (CH-AuNP) for Elemental analysis (C &D)

Table 1 — Inhibition of haemolytic activity of venom by chitosan and chitosan gold nanoparticles

OD of haemolysis *Vipera russellii* venom (VRV)(1µg)+Chitosan (15 mg/mL)	OD of Control (RBC + PBS)	OD of RBC + D. Water (100%Hemolysis)	% of Hemolysis	% Inhibition
0.375	0.04	3.675	9.11	86.72
OD of haemolysis *Vipera russellii*venom (VRV)(1µg) +CH-AuNP (2 mg/mL)	OD of Control (RBC + PBS)	OD of RBC + D. Water (100%Hemolysis)	% of Hemolysis	% Inhibition
0.104	0.04	3.675	2.82	93.01

OD=Optical density, D. Water=Distilled Water, PBS=Phosphate buffer solution, CH-AuNP= Chitosan gold nanoparticle

Table 2 — Anti-proteolytic activity of Chitosan and Chitosan gold nanoparticle (CH-AuNP) in comparison to no treatment

Groups	Proteolytic activity (Unit)	% inhibition of proteolytic activity
Vipera russellii venom (VRV)	303 ± 0.94	-
Vipera russellii venom (VRV) + Chitosan(15 mg/mL)	93 ± 0.06	68 ± 0.05
Vipera russellii venom (VRV) +CH-AuNP(2 mg/mL)	38 ± 0.02	97 ± 0.07

Table 3 — Physiological observations in Group I(saline control) and Group II mice (exposed to 15mg/kg body weight dose of chitosan)

Signs and symptoms	Group-I			Group-II		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Behavior	Normal	Normal	Normal	Normal	Normal	Normal
Skin and Fur	Normal	Normal	Normal	Normal	Normal	Normal
Eyes And mucous Membranes	Normal	Normal	Normal	Normal	Normal	Normal
Salivation	Absent	Absent	Absent	Absent	Absent	Absent
Tremors/ convulsions	Absent	Absent	Absent	Absent	Absent	Absent
Death	Nil	Nil	Nil	Nil	Nil	Nil
Other symptoms	Nil	Nil	Nil	Nil	Nil	Nil

Table 4 — Neutralization studies with chitosan gold nanoparticles (CH-AuNP)

N=6	Group II VRV(µg)	Group III VRV(µg) +CH (mg/kg; b.*w*) (Fold of protection, P/NP)	Group IV VRV(µg) +CH-AuNP (mg/kg; b.*w*) (Fold of protection)
Minimum Lethal Dose (MLD) (*i.v*)	VRV(2µg) [1MLD]	VRV (2 µg) + 15(mg/kg; b.w) (1 fold, P) VRV (4 µg) + 15(mg/kg; b.w) (2 fold, P) VRV (6 µg) + 15(mg/kg; b.w) (3 fold, NP)	VRV (2 µg) + 2(mg/kg; b.w) (1 fold, P) VRV (4 µg) + 2 (mg/kg; b.w) (2 fold, P) VRV (6 µg) + 2 (mg/kg; b.w) (3 fold, P) VRV (8 µg) + 2(mg/kg; b.w) (4 fold, P) VRV (.9 µg) + 2 (mg/kg; b.w) (4.5 fold, P) VRV (10 µg) + 2 (mg/kg; b.w) (5 fold, NP)
Minimum Hemorrhagic Dose (MHD)	VRV (10µg) [1MHD]	VRV (10 µg) + 15(mg/kg; b.w) (1 fold, P) VRV (20 µg) + 15(mg/kg; b.w) (2 fold, P) VRV (30 µg) + 15(mg/kg; b.w) (3 fold, NP)	VRV (10 µg) + 2(mg/kg; b.w) (1 fold, P) VRV (20 µg) + 2 (mg/kg; b.w) (2 fold, P) VRV (30 µg) + 2 (mg/kg; b.w) (3 fold, P) VRV (40µg) + 2(mg/kg; b.w) (4 fold, NP)

VRV: *Vipera russelli*venom ; P: indicates Protection; NP: indicates No Protection

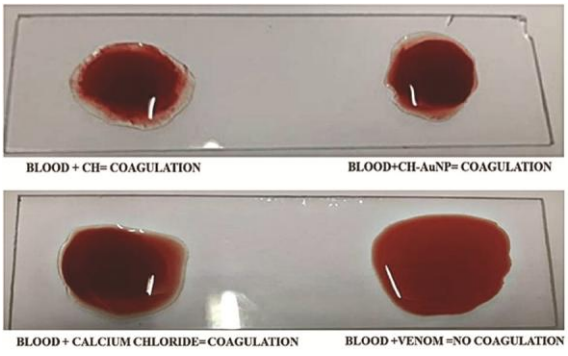

Fig. 9 — Effect of chitosangold nanoparticles (CH-AuNP) on neutralization of procoagulant activity in comparison to control

In vivo neutralization of *Vipera russelli* venom

Studies on acute toxicity

In acute toxicity studies of chitosan (exposure dosage of 15mg/kgbody weight) no abnormal changes were observed in the body weight of the test mice in comparison to the control group over a time period of 14days of exposure (Table 3).

In vivo neutralization of VRV induced lethality and haemorrhagic activity

In neutralization of lethality, chitosan neutralized up to 2 MLD of venom considered as a 2fold Protection. CH-AuNP neutralized up to 4.5 MLD of venom considered as a 4.5fold Protection. In haemorrhagic activity neutralization studies, chitosan neutralized up to 2 MHD of venom considered as a 2fold Protection. CH-AuNP neutralized up to 3 MHD of venom considered as a 3fold Protection. (Table 4 & Fig. 10).

Neutralization of nephrotoxicity

It was observed that the VRV induced increased of serum uric acid and creatinine were significantly neutralized by chitosan and CH-AuNP (Table 5 & Fig. 11).

Discussion

Recent studies have placed increasing emphasis on the use of gold nanoparticles in the field of medical

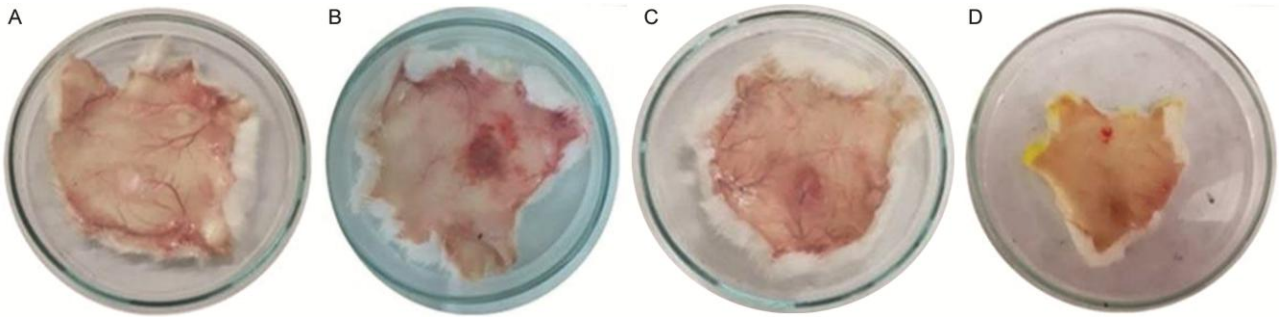

Fig. 10 — Neutralization of VRV induced haemorrhage (A) Saline control, (B) VRV treated, (C) VRV + CH treated (D) VRV + CH-AuNP treated

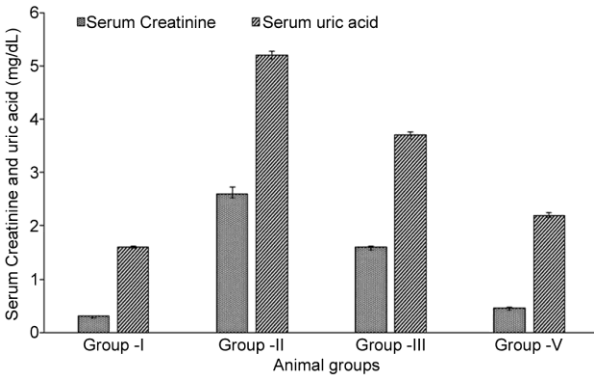

Fig. 11 — Neutralization of venom induced rise in serum uric acid and creatinine for nephrotoxicity

Table 5 — Neutralization of *Vipera russelli* venom induced nephrotoxicity markers with chitosan gold nanoparticles (CH-AuNP)

Group(s) (N=6)	Serum Creatinine(mg/dL)	Serum uric acid(mg/dL)
Group I (Sham control)	0.3±0.01	1.6±0.029
Group II (1µg VRV)	2.6±0.12[abc]	5.2±0.07[abc]
Group III (1 µg VRV+ Chitosan 15mg/kg.bw)	1.6±0.02[abc]	3.7±0.06[abc]
Group IV (1µgVRV+ CH-AuNP 2mg/kg.bw)	0.46±0.01[ac]	2.2±0.04 [ac]

[a]P< 0.05when compared Group-II with Group- III, [ab]P< 0.05 when compared Group-II with Group -IV, [ac] P< 0.05 when compare Group- III with Group- IV.

diagnostics and therapeutics[17].In the current study,UV-Visible spectroscopy analysis of gold nanoparticles exhibited a maximum absorbance peak at 530 nm.CH-AuNP demonstrated a surface Plasmon resonance at approximately 532 nm which conforms to the accepted standards[18]. Past studies have exhibited that the absorbance peak occurs due to the excitation of surface plasmon vibrations inAuNP. No other peak was observed in the UV-Vis absorption spectrum which was indicative of the complete formation of gold nanoparticles. Dynamic light scattering reports exhibited that there was formation of monodispersed nanoparticles with a hydrodynamic diameter of about 89.65 nm showing a single peak. Zeta potential of CH-AuNP was found to be +24.7 mV which specified stability of the gold nanoparticle.A past study indicated that the zeta potential provides a characteristicsign of the stability of colloidal particles. The absolute values replicated the net electrical charge on the particle's external surface that arose from the surface functional groups. The study also stated that the nanoparticles were considered to be stable colloids if their zeta potential was more than 25 mV or less than –25 mV[19].In an earlier study, X-ray diffraction analysis of AS-AuNP confirmed the cubic crystal structure of CH-AuNP. Similarly, another study stated that the antibacterial properties of chitosan gold nnaoparticles could be attributed to its crystalline structure[20].

Physical and chemical properties of AuNPs including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration, dissolution rate, particle reactivity in solution, efficiency of ion release cell type and the type of reducing agents used for synthesis have been stated as crucial factors for determination of biological activity. In a similar study conducted in the past, CH-AuNP was synthesized for drug delivery and acute and subacute toxicity wherein it was observed that all animals survived the study period without any significant variations in pathophysiological symptoms, body weight, food intake, blood parameters, organ weight and histological studies. The findings of this study demonstrated that CH-AuNP did not induce toxicity while being delivered orallytomice and have the potential for therapeutic applications[21].

The results of the current study were also similar to findings obtained from the afore mentioned study, which may indicate the therapeutic potential of CH-AuNP. In another previous study, Russell's viper venom mediated haemotixicity, nephrotoxicity, myotoxicity, and hepatotoxicity were neutralized *in vivo* by 2-hydroxy-4-methoxybenzoic acid conjugated with gold nanoparticles[22]. This was similar to the findings obtained in the present study wherein it was observed that chitosan and CH-AuNP inhibited haemotoxicity, nephrotoxicity and the proteolytic activity of VRV. Impaired haemostasis, site bleeding, myonecrosis, skin necrosis, and inflammatory response are the common symptoms observed in snake bite patients. With respect to mechanisms of lethality specific to the VRV studies have reported haemolysis, pre-synaptic neurotoxicity, rhabdomyolysis, vasodilation, and shock as major lethality factors[23]. It has also been noted that no specific dosage of anti-snake venom exists to ameliorate the side effects of venom[24]. In an earlier study, the antimicrobial and wound healing properties ofgold nanoparticles modified with chitosan was studied[25]. In another study, the chitosan coated gold nanoparticles were shown to provide low cytotoxicity and reactive oxygen species production in primary leukocytes[26]. Both AuNP and chitosan have low toxicity and high biocompatibility which ascribes to their suggested usage in nano-biotechnology. In a recent study, the effect of chitosan gold nanoparticles showed great antitumor activity against LN229 cells[27].In the present study, chitosan gold nanoparicles were observed to neutralize the VRV induced lethality, haemorrhagic activity, andnephrotoxicity, and also provide protection against venom-induced haemolysis, procoagulant activity, and proteolytic activity. Chitosan is said to possess properties such as enhanced mucoadhesion potential to interact with the mucous layer and increased drug retention time at the mucosal surface. Chitosan is also stated to possess an absorption enhancing effect due to its mucoadhesion attributes and temporary opening of the tight junctions of the mucosal cell membrane.

The capability of chitosan to boost the membrane permeability is said to be dependenton theextent of deacetylation and molecular weight *i.e.*, greater the degree of deacetylation, greater is the charge of chitosan, consequently improving the drug transportation. This is stated to aid in increased epithelial permeability. The high molecular weight of chitosan is also said to aid in increased epithelial permeability[28].In the present study, increased levels of serum uric acid and creatinine were noted in the mice exposed to VRV alone while a decrease in the markers were observed when the mice were treated with CH-AuNP.However, a vital assessment of the toxicity of chitosan NPs and its development technique needs to be studied in near future. Nano technology has gained significance in the field of biomedical sciences and research on snake venom has also been influenced by it. An earlier study, Russell's viper venom induced lethality, oedematogenic actionand phospholipase-A2 activity neutralized in animal modelwith gold nanoparticle conjugation of andrographolide[29].

Conclusion

Sometimes whole herbal extracts are less effective than the synthetic compounds. The present study has demonstrated that nanotechnology can improve the efficiency of synthetic compounds and reduce snake venom toxicity. chitosan gold nanoparticles also provided significant protection against venom induced procoagulant and proteolytic activity. CH-AuNP ameliorated the contraindication of viper venom mediated rise in nephrotoxicity markers uric acid and creatinine. In animal studies, chitosan gold nanoparticles neutralized venom induced lethality and haemorrhagic action. The findings of this investigation revealed that CH-AuNP significantly prohibited *Vipera russelli* venom-induced lethality, haemorrhagic action and nephrotoxicity in Swiss albino mice. It can be concluded that chitosan gold nanoparticles could potentially be used in a therapeutic setting to provide supplementary antivenom action against snake venom. Future studies on the synthesis methods of the nanoparticles as well as their toxicity could potentially aid in overcoming the current hurdles in antiserum therapy.

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Conflict of Interest

The authors declare no conflict of interest.

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