



Effect of *Quillaja saponaria* Molina base gold nanoparticle on Russell's viper venom toxicity

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Snakebite is a medical emergency worldwide. The primary source of saponins for industrial use is *Quillaja saponaria* Molina. The present investigation intends to explore the characterization of green synthesized gold nanoparticles and assess its potential to neutralize viper venom-induced toxicity in male albino mice. The green synthesis of AuNPs has been carried out using saponin derived from *Quillaja saponaria* Molina bark extract and the nanoparticles were characterized. FTIR, XRD, SEM, EDX, UV-visible spectroscopy and DLS studies were used to characterize the green produced SP-AuNPs. The rise of the pro-inflammatory biomarker IL-6 and TNF- α induced by *Vipera russelli* venom could considerably be inhibited by the biosynthesized SP-AuNP. The hematological study showed viper venom treatment could greatly increase the number of white blood cells and platelets, it dramatically decreased the number of red blood cells and hemoglobin. Findings demonstrated the green-synthesized AuNPs' have promising ability to protect male albino mice against the hematological and pro-inflammatory changes caused by viper venom.

Keywords: Neglected tropical diseases, *Vipera russelli* venom, Gold nanoparticle, *Quillaja saponaria* Molina

Therapeutic potential of nanoparticles for disease prevention and treatment has been emphasized by advancement in the field of nanotechnology. Herbal based metal nanoparticles have emerged as a promising tool in medicine with applications spanning drug and gene delivery¹. Traditional methods for synthesizing of gold nanoparticles often employ harmful chemicals, which can have detrimental effects on human health and the environment. Green synthesis techniques provide a solution to the environmental and health issues associated with conventional methods. By utilizing natural metabolites from plants, microorganisms, and algae this approach enables the production of environmentally safe and affordable nanoparticles^{2,3}. Phytochemical constituents help in the stabilization and reduction of metal ions during the green synthesis of nanoparticles^{4,5}. Several studies have reported in earlier a variety of plants and plant parts used in metal nanoparticle generation, the bark of *Mimusops elengi* was used to synthesize gold nanoparticles were synthesized using *Azadirachta indica* leaf extract and *Aelovera* plant extract and lemongrass extract⁶. Green

synthesis has emerged as a vital approach to address environmental issues, using safe and renewable materials for nanoparticle synthesis. This study demonstrates the use of *Quillaja saponaria* Molina bark extract saponin for the eco-friendly production of gold nanoparticles. *Quillaja saponaria* Molina tree bark represents the main source of *Q. saponaria* saponin that has anti-inflammatory and immuno stimulant effects⁷.

Quillaja saponaria Molina bark saponin is a triterpenoid saponin complex in which the sapogenin (aglycone) is a triterpene termed quillaic acid⁸. *Quillaja* bark saponin is characteristically different from other triterpenoid plant saponins regarding chemical structure. The difference may be represented by a fatty acid domain and a triterpene aldehyde group at carbon-4 of the triterpene⁹.

The present study aimed to investigate the venom-induced changes in haematological and inflammatory markers following viper envenomation. Envenomation is a severe, atypical state resulting from an overwhelming infection that causes low blood pressure and reduced blood flow¹⁰. Serious side effects, including multiple organ failure and disseminated intravascular coagulation, may manifest in the victims. Numerous studies have been conducted on cytokine production in envenomation and it

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appears that sepsis syndrome is associated with an excess of pro-inflammatory cytokines¹¹.

A complete blood count is vital for assessing blood cell types and numbers. Analyzing hematological parameters that help to detect and diagnosing disease conditions, including anemia, infections and other blood-related disorders.

Therefore, the present study has been focused on the characterization of green synthesized gold nanoparticles using saponin from bark extract of *Quillaja saponaria* Molina and to evaluate whether this herbal nanoparticle can increase the efficiency of herb for alteration of hematological indices as well as pro-inflammatory marker against *Viper russelli* venom induced toxicity in male albino mice.

Materials and Methods

Chemicals

Ethylene diamine tetra acetic acid (EDTA) and Chloroauric acid (HAuCl₄) were procured from Sigma Aldrich (Catalogue number: 484385).

Green synthesis of AuNPs using saponin bark extract from *Quillaja saponaria* Molina

Saponin from *Quillaja saponaria* Molina was procured from Sigma Aldrich. 50 mL of deionized water was used to dissolve 100 mg of the powdered bark sample. 40 mL of bark extract and 10 mL of a 1 mM HAuCl₄ solution were combined for the green synthesis of AuNP and the mixture was agitated for 4-h at 70°C. A visual inspection confirmed the shift in the colour combination. The powdered green synthesised AuNPs was then placed in an airtight container for storage¹².

Characterization of green synthesized AuNPs

The optical properties of green-synthesized gold nanoparticles were characterized by measuring their Surface Plasmon Resonance absorption band using an UV-Visible Spectrophotometer with a focus on the 400-800 nm spectral range¹³. The morphology and shape of green synthesized AuNPs were characterized using scanning electron microscope (SEM)¹⁴. Furthermore, EDX spectroscopy was used to determine the elemental composition and quantify the proportions of the nanoparticles. Fourier transform infrared spectra (FT-IR) of saponin from *Quillaja saponaria* Molina bark extract and green synthesized AuNPs was measured using with scanning range between 4000 to 500 cm⁻¹¹³. X-ray diffraction pattern was operating at a voltage 30 kV and current 10 mA with Cu-K α radiation to confirm the crystalline nature

of green synthesized AuNP¹⁴. Dynamic light scattering (DLS) measurements were conducted using a Zetasizer to determine the size distribution and dispersity of nanoparticles.

Selection of animals

Healthy male albino mice (18 \pm 2gm) were obtained from authorized animal suppliers of Vidyasagar University. Animals were kept in polypropylene cages then maintained within our animal facilities and allowed food and water *ad libitum*. The study complied with protocols approved by the Animal Ethical Committee (VU/IAEC/CPCSEA/07/06/2022) and CPCSEA regulations.

Treatment protocol

The mice were divided into five groups each group having six mice (n = 6) Group-I: Control; Group- II: received intravenous injection of *Vipera russelli* venom (1 μ g), Group-III: received intravenous injection of *Vipera russelli* venom (1 μ g) along with co-administration of saponin from *Quillaja saponaria* Molina (4mg/kg *b.w*), Group-IV: received intravenous injection of *Vipera russelli* venom (1 μ g) along with co-administration of green synthesized AuNPs (0.65 μ g/kg *b.w*), Group-V: received intravenous injection of *Vipera russelli* venom(1 μ g) along with AVS (2 mg/mL) (50 μ L). Animals were euthanized using chloroform inhalation, and blood samples were collected for hematological and serum-inflammatory biomarker analysis. For hematological and serum-inflammatory biomarker evaluation, whole blood was collected into EDTA containing polypropylene tubes.

Estimation of haematological parameters

Different haematological parameters such as red blood cells (RBCs), white blood cells (WBCs), hemoglobin (HB), platelets (PLTs) were performed on semi-automatic hematology analyzer (Abacus Vet 5, Diatron). Haematological parameters were performed by using 25 μ L whole blood samples.

Estimation of serum inflammatory cytokines

The levels of cytokines IL-6, and TNF- α in the serum from male albino mice were assayed by a two-site sandwich enzyme-like immunosorbent assay (ELISA) using pairs of monoclonal antibodies¹⁵. Serum inflammatory cytokines were measured using an ELISA reader at 540 nm. Each sample was assayed in duplicate and concentrations (pg/mL) were calculated from standard curves run in parallel.

Statistical analysis

Statistical analysis was performed using the Statistical package for social Science programme (SPSS version 15). Values were expressed as mean \pm standard error of mean, $n=6$ and $P < 0.05$ was considered statistically significant. Comparison was done between the means of control and with experimental groups, by one way analysis of variance (ANOVA).

Results

Characterization of green synthesized AuNPs

Colour Change

Green synthesized gold nanoparticle production was shown by this preliminary phytochemical analysis. Aqueous bark extract of *Quillaja saponaria* Molina saponin was added to chloroauric acid solution and within 4-h the colour changed from pale yellow to ruby red due to the reduction of gold ions and excitation of surface plasmon oscillation caused by electromagnetic field (Fig. 1).

UV-Visible spectral analysis

Surface Plasmon Resonance (SPR) a size-dependent quantum mechanical phenomena, is responsible for the broad absorbance peak centred at 530 nm in the UV-visible spectral analysis (Fig. 2)¹⁶. The generation of green synthesized AuNPs in the solution is confirmed when the SPR bands are centred between 500 and 700 nm.

Dynamic light scattering analysis

DLS analysis was used to measure the particle size and dispersity of AuNPs generated and capped with an aqueous bark extract of saponin from *Quillaja saponaria* Molina¹⁷. The DLS analysis of SP-AuNP in this study revealed a single peak in the size distribution histogram and its hydrodynamic diameter was obtained at 52.38 nm. The surface charge of the particles is revealed by the zeta-potential of a colloidal solution. The zeta potential of SP-AuNP was (-6.25 mV) (Fig. 3).

FT-IR analysis

FT-IR revealed peaks in the range of 3425 cm^{-1} (O-H stretching), 2943 cm^{-1} (C-H stretching), 1733 cm^{-1} (C=O stretching), 1639 cm^{-1} (C=C stretching), 1073 cm^{-1} (S=O stretching), 646 cm^{-1} (=C-H bending) in saponin. FT-IR spectra of SP-AuNP showed peaks at 458 cm^{-1} representing a Alkenes (-C=H-) group. The peaks 1031 cm^{-1} showed (C=O) and Aldehyde (CHO) groups. The peaks



Fig. 1 — Colour Change during phytochemical synthesis of chloroauric acid to ruby red colour.

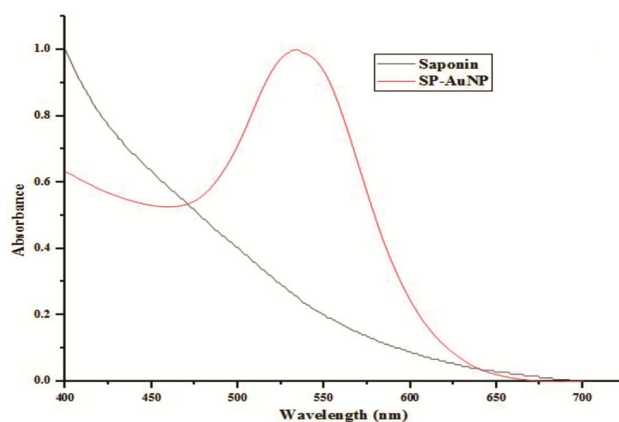


Fig. 2 — UV-Vis absorption spectrum of Saponin(SP) and gold nanoparticle of saponin(SP-AuNP).

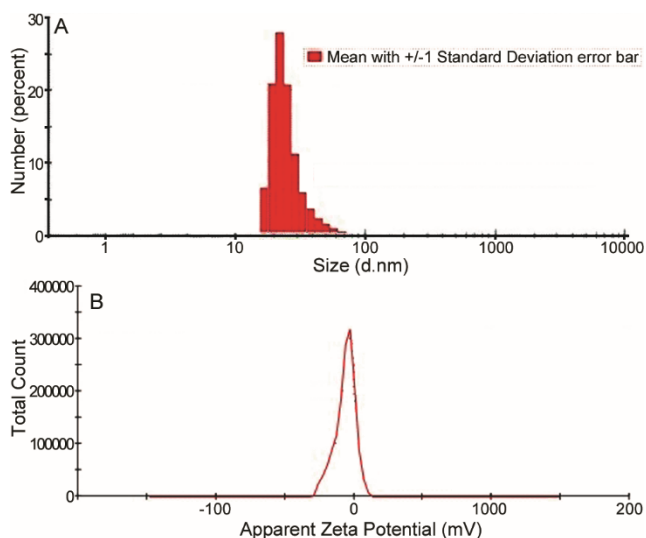


Fig. 3 — (A) The hydrodynamic size and (B) Zeta potential of SP-AuNP.

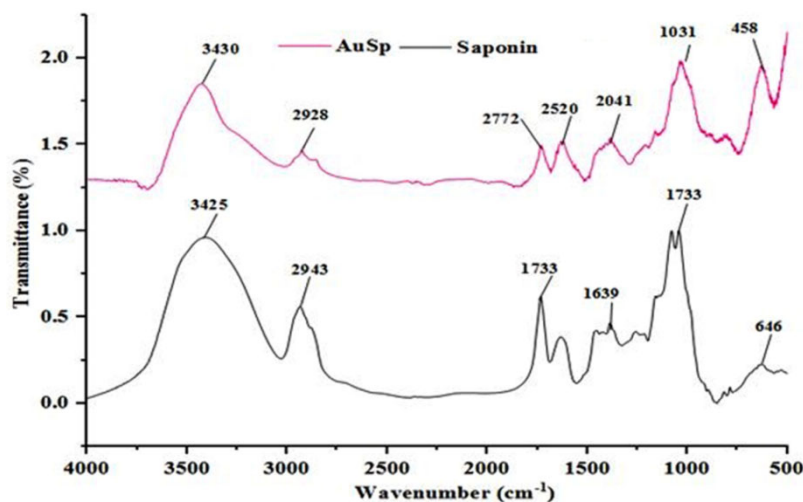


Fig. 4 — The FT-IR spectrum of SP and SP-AuNP.

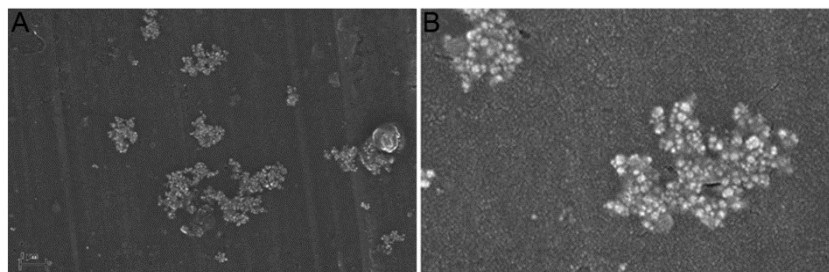


Fig. 5 — SEM Micrographs of SP- AuNPs.

2928 cm^{-1} and 3430 cm^{-1} represents Alkanes (C-H) and Phenols (O-H)/amines group (Fig. 4).

SEM and Energy Dispersive X-Ray (EDX) analysis

The SEM study of green synthesized AuNPs confirmed that particles were mostly spherical in nature and had sizes less than 40 nm (Fig. 5). The size distribution study also revealed that nanoparticles were well dispersed. Analysis through energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental gold signal of the gold nanoparticles. A strong signal was obtained from the gold atoms in the nanoparticles at 2 keV, and a weak signal was observed from “K” and “O.” This analysis revealed that the nanostructures formed were solely of gold (Fig 6).

X-ray diffraction pattern analysis

The X-ray diffraction analysis was performed to confirm the crystalline nature of SP-AuNP (Fig. 7) The X-ray diffraction analysis was performed to confirm the crystalline nature of SP-AuNPs. The diffraction peaks at 2θ values of 38.2°, 44.4°, 64.7°, 77.7°, 81.9°. X-ray diffraction pattern of AuNP-SP which confirms the face-centered cubic (FCC) XRD spectrum of SP-AuNPs displayed a distinct crystalline structure of gold.

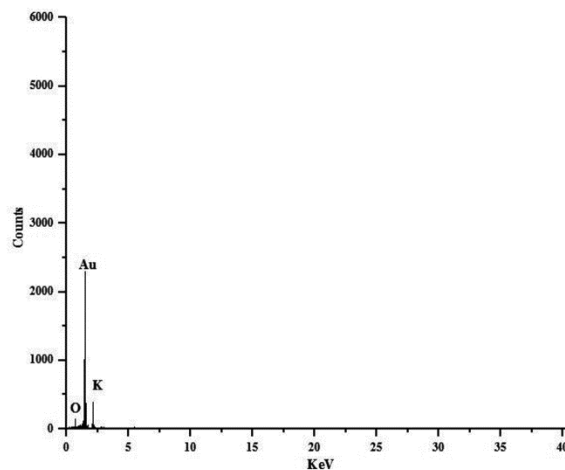


Fig. 6 — EDX analysis of SP- AuNPs.

Thermogravimetric analysis (TGA)

The thermogravimetric analysis revealed a significant weight loss (%) between 40-900 °C (Fig. 8). This reduction in weight (%) occurred linearly with the increase in temperature. This decrease indicated that at higher temperatures, the molecules from *Quillaja saponaria* Molina that surround the gold nanoparticles, acting as the capping and stabilizing components for the nanoparticles,

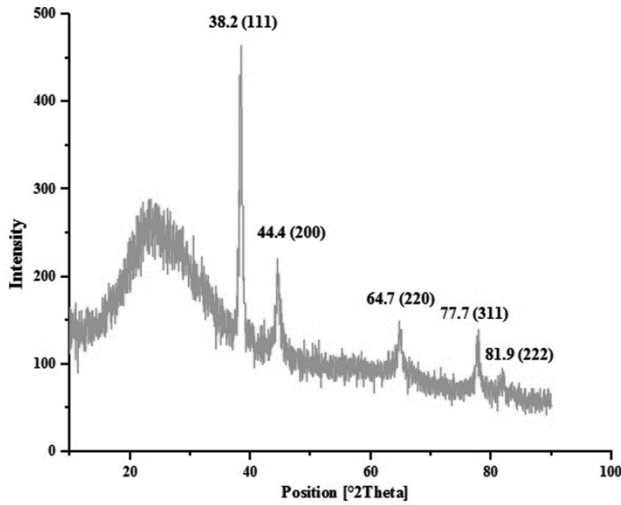


Fig. 7 — XRD analysis of SP- AuNPs.

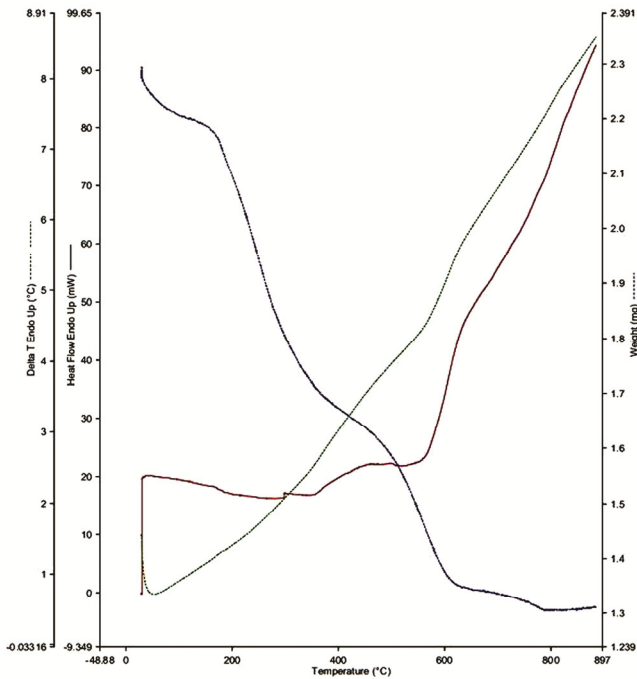


Fig. 8 — TGA analysis of SP- AuNPs.

were completely degraded. Hence, this serves as confirmation that the gold nanoparticles synthesized from *Quillaja saponaria* Molina were capped with organic compounds.

Effect of green synthesized AuNPs on hematological parameters
 The present study was conducted to evaluate the protective role of green-synthesised AuNPs against *Vipera russelli* venom-induced alterations in haematological parameters in male albino mice. The study revealed that green synthesized AuNPs when co-administered with viper venom at 0.65 µg/kg body weight conferred protective benefits. Intravenous

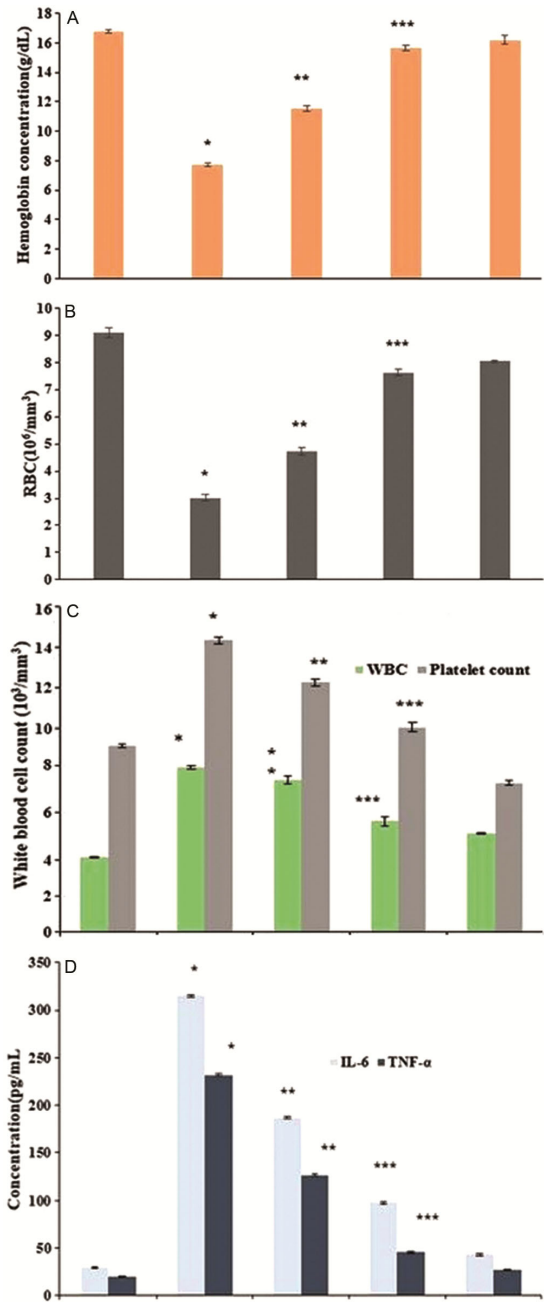


Fig. 9 — Effect of saponin from *Quillaja saponaria* Molina bark extract and green synthesized AuNPs on (A) hemoglobin level; (B) red blood cell count; (C) white blood cell count, (D) serum inflammatory cytokines in mice after intravenous administration of *Vipera russelli* venom. * $P < 0.05$ when compared Gr. II with Gr. III, ** $P < 0.05$ when compared Gr. II with Gr. IV, and *** $P < 0.05$ when compared Gr. II with Gr. V] Gr I: control, Gr II: Viper russelli venom, Gr III: Viper Russell venom + saponin, Gr IV: *Vipera russelli* venom + green synthesized AuNPs., GrV: *Vipera russelli* venom + AVS.

administration of viper venom caused a marked reduction in red blood cell count, in contrast to the control group. (Fig. 9A) (Fig. 9B) (Table 1). The white

Table 1 — Effect of green synthesized SP-AuNPs on hematological parameters

Animal group	Hemoglobin concentration (g/dL)	RBC ($10^6/\text{mm}^3$)	WBC ($10^3/\text{mm}^3$)	Platelet ($10^3/\text{mm}^3$)
Group-I	16.75 ± 0.125	9.09 ± 0.174	4.13 ± 0.04	8.80 ± 0.06
Group-II	7.74 ± 0.135*	3.02 ± 0.110 *	8.89 ± 0.07*	14.25 ± 0.151*
Group-III	11.51 ± 0.179**	4.72 ± 0.128**	7.38 ± 0.180**	11.46 ± 0.145**
Group-IV	15.62 ± 0.176***	7.62 ± 0.109***	5.65 ± 0.192***	9.59 ± 0.197***
Group-V	16.19 ± 0.274	8.03 ± 0.04	5.12 ± 0.02	7.26 ± 0.09

[Values were expressed as mean ± standard error of mean, n=6; * $P < 0.05$ when compared Gr. II with Gr. III, ** $P < 0.05$ when compared Gr. II with Gr. IV, and *** $P < 0.05$ when compared Gr. II with Gr. V] Gr I: control, Gr II: Viper russelli venom, Gr III: Viper Russell venom + saponin, Gr IV: *Vipera russelli* venom + green synthesized AuNPs., GrV: *Vipera russelli* venom + AVS. RBC: Red blood cell; WBC: White blood cell]

blood cell was most reduced when treated with green synthesized AuNPs (Fig. 9C) (Table 1).

Effect of green synthesized AuNPs on serum inflammatory cytokines

Serum inflammatory cytokines were determined as above described and to investigate the changes in serum levels of proinflammatory cytokines (IL-6 and TNF- α). A substantial increase in TNF- α and IL-6 levels was observed after administration of *Vipera russelli* venom. Treatment with saponin extracted from *Quillaja saponaria* Molina and green-synthesized gold nanoparticles effectively modulated these elevated pro-inflammatory cytokine levels (Fig 9D).

Discussion

Nanotechnology is an upcoming technology which has tremendous potentials in biomedical research. Nanoparticles have remarkable properties that can be used to improve the pharmacological and therapeutic properties while tagged natural biomolecules including venoms and toxins¹⁸. In the present studies, physicochemical and structural features of SP-AuNP were investigated using dynamic light scattering (DLS), Fourier-transform Infrared (FTIR), scanning electron microscopy and X-ray diffraction (XRD) techniques. Green synthesized AuNPs conferred protective benefits when co-administered with viper venom at 0.65 $\mu\text{g}/\text{kg}$ body weight. The DLS analysis of SP-AuNP in this study revealed a single peak and its hydrodynamic diameter was obtained at 52.38 nm. The surface charge of the particles is revealed by the zeta-potential of a colloidal solution¹⁹. In earlier study, X-ray diffraction analysis of gold nanoparticles which confirmed the cubic crystal structure of SP-AuNP²⁰. The Fourier-transform infrared spectroscopy investigation revealed various functional groups from saponins that contribute to the stability and capping of AuNPs²¹. In the present study, a similar pattern of FTIR spectra was observed for saponins and SP-

AuNP which confirms the synthesis and capping process of AuNP using crude saponins. Further confirmation of the successful synthesis of AuNPs was provided by scanning electron microscopic analysis that showed 15-40 nm in size. As reported earlier, saponin gold nanoparticles were prepared using *Sapindus mukorossi* extract²². The various components of the venom cause hematological changes, which may be linked to concurrent and intricate pathogenic pathways²³. This study found that the intravenous administration of *Vipera russelli* resulted in a significant reduction in red blood cell count compared to the control group. Red blood cell degeneration and a decrease in the rate of erythropoiesis are indicated by a decrease in the number of red blood cells.

When green synthesized AuNPs were administered concurrently with viper venom the count of red blood cells revealed a considerable rise in comparison to the albino mice that received viper venom treatment. Erythropoietin is the maturation factor that promotes the production of red blood cells from hemopoietic stem cells in the bone marrow. Hence, changes in the number of red blood cells may be attributed to the hematopoietic system²⁴.

In comparison to control, venom treated group demonstrated a considerable rise in white blood cells following the administration of viper venom. However, when green synthesized AuNPs were co-administered with viper venom and saponin from *Quillaja saponaria* Molina bark extract treated mice WBC levels significantly decreased.

White blood cells are immune system cells that play a role in warding off infections. Hence, green synthesized AuNPs revealed effective protection of WBCs during infections. Hemoglobin level and red blood cell decreased due to the effect of the venom compared to group iii, which received intravenous injection of *Vipera russelli* venom along with co-

administration of saponin from *Quillaja saponaria* Molina and group iv, which received intravenous injection of *Vipera russelli* venom along with co-administration of green synthesized AuNPs. White blood cells are effectors of the immune system significant changes were observed in venom treated group and green synthesized AuNPs treated group extract. This suggests that the green synthesized AuNPs must have combated the venom directly without cells of the immune system producing effectors cells. Pathological properties of Russell's viper venom are mainly associated with hematological disturbances. The intention of the current investigation was to determine if green-synthesised AuNPs could protect male albino mice from the hematological changes caused by viper venom. In earlier similar study performed using bee venom the results of the present study are consistent with those²⁵. Serum inflammatory cytokines IL-6 and TNF- α were found to increase in viper venom treated group of Albino mice while treatment with green synthesized AuNPs significantly reduced confirming the anti-inflammatory effects of SP-AuNPs. Earlier in a similar study, titanium dioxide nanoparticles was investigated which showed excellent the anti-inflammatory effects against Viper and Cobra²⁶. Present study further supported by previous reports showing that *Quillaja saponins* can induce the direct activation of antigen-presenting cells as well as expression of cytokines such as IL-1 β , IL-6, IL-8, IL-18, IL-12, and TNF- α even in the absence of pathogens²⁷. Pro-inflammatory cytokines are principally responsible for generating a strong defense against external infections. But shock, multiple organ failure and mortality may be considerably exacerbated by an overproduction of these mediators²⁸. The systemic inflammatory response, mediated by cytokines such as TNF- α and IL-6 plays a crucial role in envenomation. Elevated levels of these cytokines in envenomated mice correlate with the severity and outcome of the condition. Present study aims to investigate the balanced ratio of pro-inflammatory and anti-inflammatory cytokines is essential for an appropriate immune response, as excessive inflammation or hyporesponsiveness can lead to Russell's viper venom. The mechanism of action of SP-AuNP induced venom toxicity neutralization need further attention, for the development of therapeutic antagonist against viper venom toxicity.

Conclusion

The present study is an attempt to investigate the potential of SP-AuNP in neutralizing viper venom

induced changes in hematological and inflammatory markers. This herbal reduction approach can be rapidly and easily scaled up for large-scale synthesis, and it is also quite affordable. Therefore, supplementing with green synthesized AuNPs may be a safe therapeutic strategy for prevention of snake envenomation.

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Conflict of interest

The authors declare no conflict of interest.

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