Russell’s viper venom induced nephrotoxicity, myotoxicity, and hepatotoxicity—Neutralization with gold nanoparticle conjugated 2-hydroxy-4-methoxy benzoic acid in vivo

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Snakebite is one of the major neglected tropical diseases and health hazard that leads to significant mortality, particularly in rural populations of tropical and subtropical countries including India. Antisnake venom serum (ASVS) is the only specific treatment against snake envenomation. Available treatment i.e. ASVS have many limitations not only low efficiency but also considerable side effects. Search for alternative ASVS is a major domain in toxinology research. Targeted drug therapy using nanoparticles, an emerging area of nanotechnology, is one such alternative. Here, we studied neutralization of ing Russell’s viper venom (RVV) induced toxicity (nephrotoxicity, myotoxicity, hepatotoxicity) with gold nanoparticle-conjugated 2-hydroxy-4-methoxy benzoic acid (GNP-HMBA) in male albino mice. We conjugated 2-hydroxy-4-methoxy benzoic acid (HMBA) with gold nanoparticle (GNP) by adsorption method, and physico-chemical characterization were done by DLS, ZETA potential, FTIR and TEM. Swiss male albino mice were divided into four groups viz., sham control, venom control, HMBA treated and GNP-HMBA treated. Each group had four mice (n=4). RVV was injected in all groups except sham control. Groups 3 and 4 had treatment with HMBA and GNP-HMBA, respectively. After 24 h, blood and urine were collected. Serum LDH, CK, SGPT, SGOT, γ-GT, ACP, ALP, urea, creatinine and urinary calcium and urinary phosphorus were measured. The hydrodynamic diameter of GNP-HMBA was 65-75 nm and TEM diameter was 18-28 nm. The serum/urine parameters were found significantly increased in venom control group. Degree of RVV neutralization was GNP-HMBA > HMBA. Treatment with GNP-HMBA showed partial protection of histopathological changes in RVV-induced kidney and liver tissues. It may be concluded that GNP-HMBA neutralized RVV-induced toxicities (nephrotoxicity, myotoxicity and hepatotoxicity) in male albino mice. Further studies are warranted in the development of alternative herbal-nanoparticle antidote against snake venom induced toxicity.

Keywords: Antisnake venom serum (ASVS), Daboia russelli russelli, HMBA, Snakebite, Snake venom, Viperidae

Snakebite is a neglected tropical disease and a major health hazard with high mortality rate among rural populations of tropical and subtropical countries, especially India. World over, there are about 3150 species of snakes and around 600 of them are venomous. About 216 species of snakes are reported to occur in India, of which 60 are considered poisonous. The incidence of snake bite lethality is high in sub-Saharan Africa, Asia, and Latin America. In India, the range widely varies from 1300 to 50000 deaths per year mostly involving four (‘Big Four’) snakes, Russell’s Viper (Daboia russelii), Spectacled Cobra (Naja naja), Common Krait (Bungarus caeruleus) and Saw-scaled Viper (Echis carinatus). Till now, the antisnake venom serum (ASVS) immunotherapy is the only specific treatment against snakebite. It is pertinent to note that there is no antivenom available in India for the bites of King Cobra, sea snakes and few species of pit viper and also the available Indian antivenom, due to geographic and species variation, comparatively has limited potency. Further limitations viz., shelf-life, cost, nil protection against local effects, hemorrhage and organ toxicity, and the side effects such as anaphylactic shock, pyrogenic reaction, serum sickness, etc. of the commercially available ASVS are also well known.

Over the years, several attempts have been ventured for the development of alternative snake venom antagonists from herbal resources. Many medicinal plants, which appear in old drug recipes/traditional medicine, are possible snakebite antidote and are recommended for the treatment of snakebite. In almost any part of the world, where
venomous snakes occur, numerous plant species are used as folk medicine to treat snakebite. Indian Sarsaparilla (*Hemidesmus indicus*, Asclepiadaceae) or *anantamul* is a herb found in South Asia. The roots of *H. indicus* are used to treat snake bite and scorpion sting. An organic acid, 2-hydroxy-4-methoxy benzoic acid (HMBA) isolated and purified from the root extract of *H. indicus*, has been shown to possess viper venom inhibitory activity. In Ayurveda, herbs are conjugated with metal (gold, silver, iron, etc.) ashes to increase the efficacy of herbs. Gold nanoparticles and nano based products are presently employed in the field of biomedicine and have shown promising advantage as drug molecule. From the present laboratory, gold nanoparticle was conjugated with HMBA and its antiviper venom activity (lethality, edema, necrosis and PLA2) was established in animal model.

Russell’s viper (Viperidae) is common in the eastern India, particularly in West Bengal. Clinical symptoms after Russell’s viper bite is characterized by hemorrhage, local tissue damage, nephrotoxicity and myotoxicity. Among the several clinical manifestations of viper bite, organ toxicity is one of the major causes of death. Cardiovascular shock and multi organ failure may occur due to coagulopathies, bleeding, renal and hemodynamic alterations, which are characterized by viperidae snakebite. Acute myocardial infarction was reported in patients with Russell’s viper bite. Hepatotoxicity was also reported after Russell’s viper bite. Viperidae phospholipase A2 (PLA2) induced acute muscle damage at the site of venom injection has been reported. ASVS failed to neutralize the above pathophysiological changes especially the organ toxicity.

In the present study, we tried conjugating gold nanoparticle with 2-hydroxy-4-methoxy benzoic acid (HMBA), characterize its physicochemical property and explore the viper venom induced toxicity (nephrotoxicity, myotoxicity and hepatotoxicity) neutralization with GNP-HMBA in male albino mice.

**Materials and Methods**

**Venom**

Lyophilized snake venom from *Daboia russelli russelli* was commercially collected from Calcutta Snake Park, Kolkata, India and preserved in desiccators at 4°C in an amber coloured glass vial until further use. The snake venom was dissolved in Milli-Q water, kept at 8°C for 18 h and centrifuged at 3000 rpm for 10 min. The supernatant was used as venom and kept at 8°C until further use. The venom concentration was expressed in terms of dry weight (mg/mL, w/v).

**Herbal compound**

The 2-hydroxy-4-methoxy benzoic acid (HMBA) was obtained commercially from Sigma-Aldrich (USA). Before use, the compound was dissolved in Milli-Q water at 60-70°C. The concentration of HMBA was expressed in terms of dry weight (mg/mL, w/v).

**Animals**

Swiss male albino mice (20±2 g) aged 8 wk were obtained from authorized animal suppliers of Calcutta University. The animals were kept in polypropylene cages, acclimatized and maintained in a controlled environment (temperature, 25±2°C; humidity, 60±5%; and 12 h light/dark cycle). Food (pellet diet, Bengal gram and fresh green vegetables) and water were provided in *ad libitum*. All experimental protocols described in this study were approved by the animal ethics committee, Dept. of Physiology, University of Calcutta (animal ethical clearance no: IAEC/Revised Proposal/AG01/2012 dt.01.02.2013) and were in accordance with the guideline of the committee for the purpose of control and supervision of experiments on animal (CPCSEA), Government of India.

**Gold nanoparticle conjugation with HMBA (GNP-HMBA)**

Gold salt was purchased from Sigma-Aldrich (USA). Gold nanoparticle was synthesized by citrate reduction method with slight modifications. About 50 mL of Milli-Q water was heated to 80°C and stirred at 400-450 rpm, and 1250 µL of 10 mM HAuCl₄ was added to it with continuous stirring followed by addition of 1500 µL of 10 mM Sodium citrate and stirred continuously for 30 min. After the formation of gold nanoparticle (GNP), it was centrifuged at 30000 rpm for 30 min and was re-suspended in Milli-Q water.

Gold nanoparticle was conjugated with 2-hydroxy-4-methoxy benzoic acid (GNP-HMBA) by adsorption method. The 2-hydroxy-4-methoxy benzoic acid (dissolved in Milli-Q water) was added in the freshly prepared gold nanoparticle at the ratio of 3:8 and was kept at 37±1°C for 48 h and further kept at room temperature (25±3°C).

**Characterization of GNP and GNP-HMBA**

Hydrodynamic diameter of GNP-HMBA was determined by dynamic light scattering (DLS) using...
Beckmann Coulter Delsa Nano C TM. Stabilization of GNP-HMBA was determined by zeta potential. FTIR spectrum was obtained using Nicolet 6700-FTIR. The size, shape and morphology of GNP-HMBA were determined by transmission electron microscopy by using JEOL JEM 2100.

**Experimental design**

Swiss male albino mice (20±2 g) were divided into four groups viz., Gr. I with sham control, Gr. II with venom control, Gr. III with HMBA treated; and Gr. IV with GNP-HMBA treated. Each group had four mice (n=4). Russell’s viper venom (RVV) @ 10 µg was injected (s.c.) in groups II-IV. HMBA (100 µg) and GNP-HMBA (200 µL) were injected (i.v.) in groups III & IV animals, respectively after 1 h of RVV injection. After 24 h, blood/urine were collected for analysis.

**Neutralization of toxicity (nephrotoxicity, myotoxicity, hepatotoxicity)**

In RVV-induced nephrotoxicity study, urine phosphorus was determined after Fiske and Subbarow method\(^{17}\), and was treated with acid molybdate reagent to produce phosphomolybdic acid from any inorganic phosphate present in the urine. The phosphomolybdic acid was reduced by 1,2,4-amino napthol sulphonic acid reagent to produce a blue colour, whose intensity was proportional to the amount of phosphorous present in the urine. The absorbance of the samples were taken against blank at 660 nm using spectrophotometer (Analab UV-180). Urinary calcium was measured by biochemical kit (Merck, India) according to the manufacturer’s instruction manual. Serum creatinine, urea were measured by biochemical kit (Merck, India) according to the manufacturer’s instruction manual.

In RVV-induced myotoxicity study, serum lactate dehydrogenase (LDH) and creatine kinase (CK) were determined by using biochemical kit (Merck, India) according to the manufacturer’s instruction manual.

In RVV-induced hepatotoxicity study, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), γ-glutamyl transferase (γ-GT), acid phosphatase (ACP) and alkaline phosphatase (ALP) were estimated by biochemical kit (Merck, India) according to manufacturer’s instruction manual.

**Histopathological studies**

Kidney and liver tissues were collected from respective groups after acute exposure of RVV (10 µg/20 g/s.c.), 10% buffered formalin was used for fixation. Graded ethanol (50-100%) was used for dehydration followed by clearing in xylene. Tissues were then embedded in paraffin (56-58°C) at 58±1°C for 4 h. Paraffin sections were deparaffinized with xylene, stained with haematoxylin–eosin, followed by mounting in DPX with a cover slip. Histological changes were observed with a bright field microscope (Motic, Germany) and photographs were captured (Motic Images Plus 2.0 software) for documentation (magnification 150X).

**Statistical analysis**

Statistical analysis was done by one way analysis of variance (ANOVA). Values were expressed as mean ± standard error of mean (n=4) and \( P <0.05 \) was considered statistically significant.

**Results**

**Formation & characterization of GNP-HMBA**

The colour of GNP-HMBA was pink and was stable up to 90 days at 8±2°C. Dynamic light scattering data revealed the formation of monodispersed nanoparticles both in case of GNP and GNP-HMBA. The hydro-dynamic diameter of GNP and GNP-HMBA was 30-40 nm and 65-75 nm, respectively with polydispersity index of 0.499 and 0.375 (Fig. 1 A and B). Zeta potential of GNP and GNP-HMBA was −20.02 mV and +16.80 mV, respectively (Fig. 2 A and B).

![Fig. 1—Dynamic light scattering (DLS). (A) GNP; and (B) GNP-HMBA. [The hydrodynamic diameter of GNP was found to be 30-40 nm with polydispersity index of 0.499. The hydrodynamic diameter of GNP-HMBA was found to be 65-75 nm with polydispersity index of 0.375]](image-url)
Analysis of FTIR spectra revealed the inclusion of the post lanthanide late transitional element gold as a part of the complex. The stretching of the curve at 3431.65 cm\(^{-1}\) revealed the presence of free \(-\text{OH}\) in the solution. There was another stretching at the position of 1602.69 cm\(^{-1}\) which indicated the presence of \(-\text{COOH}\) group in the GNP solution (Fig. 3A). FTIR spectrum of GNP-HMBA stretching at the 3439.85 cm\(^{-1}\) revealed that there was presence of free \(-\text{OH}\) group which may come from the HMBA and another small stretching at 2929.60 cm\(^{-1}\) indicated the presence of aromatic group. Presence of free carboxylic group in the GNP-HMBA solution was confirmed by the stretching of spectrum at 1631.90 cm\(^{-1}\) confirming the conjugation of GNP with HMBA (Fig. 3B). Transmission electron microscopy showed spherical GNP and GNP-HMBA with particle size 15-20 nm and 18-28 nm, respectively (Fig. 4A and B).

Neutralization of RVV induced nephrotoxicity by GNP-HMBA

RVV (10 µg/20 g/s.c.) significantly \((P<0.05)\) increased serum creatinine, urea and urinary calcium, phosphorus levels in Gr. II mice when compared to Gr. I sham control mice. GNP-HMBA (200 µL/20 g/i.v.) significantly decreased \((P<0.05)\) serum creatinine, urea and urine calcium and phosphorous levels in Gr. IV mice when compared to Gr. II venom control mice. HMBA (100 µg/20 g/i.v.) treated Gr. III animals also showed significant \((P<0.05)\) protection against RVV induced nephrotoxicity when compared to Gr. II venom control mice. The degree of protection offered by GNP-HMBA was higher than that of HMBA (Table 1 & Fig. 5).
In venom control Gr. II animals, serum creatinine level was significantly higher (P < 0.05) (0.91±0.01 mg/dL) as compared to sham control group I animal (0.26±0.01 mg/dL), which indicated venom induced nephrotoxicity. Treatment with GNP-HMBA significantly (P < 0.05) decreased serum creatinine level (0.17±0.02 mg/dL) as compared with HMBA treated mice (0.36±0.01* mg/dL). The degree of protection offered by GNP-HMBA was more than that of HMBA (Table 1)

Serum urea level was significantly (P < 0.05) high in venom control Gr. II mice (49.70±1.37 mg/dL) when compared to Gr. I sham control mice indicating RVV induced nephrotoxicity. Treatment with GNP-HMBA significantly (P < 0.05) decreased serum urea level (0.17±0.02 mg/dL) as compared with HMBA treated mice (0.36±0.01 mg/dL). The degree of protection offered by GNP-HMBA was more than that of HMBA (Table 1).

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The urinary calcium and phosphorous levels in venom control Gr.II mice were significantly Higher (3.06±0.12 and 2.20±0.11 mg/dL respectively) compared to Gr. I control animals which indicated increased risk of acute renal damage in RVV induced mice. Treatment with GNP-HMBA significantly (P < 0.05) decreased urinary calcium and phosphorous level (1.97±0.11 and 0.68±0.10 mg/dL respectively) in Gr. IV animals as compared to Gr. II animals. Treatment with HMBA in Gr. III animals caused no significant change compared to Gr. II animals. GNP-HMBA gave more protection than HMBA against RVV induced nephrotoxicity in male albino mice (Fig. 5).

RVV induced histopathological changes in kidney tissue included congested glomerulus, cellular infiltration, inflamed and enlarged renal tubules with presence of blood. Treatment with GNP-HMBA showed partial recovery (less glomerular congestion, less cellular infiltration, less inflamed enlarged renal tubule without presence of blood) in kidney tissue histology (Fig. 6).

Neutralization of RVV induced myotoxicity by GNP-HMBA

RVV (10 µg/20 g/s.c.) significantly (P < 0.05) increased serum LDH level in Gr. II venom control mice (433.52±27.45 U/L) as compared with Gr. I sham control mice (156.19±10.00 U/L), Gr. III HMBA treated mice (186.61±34.12 U/L) and Gr. IV GNP-HMBA treated mice (196.56±11.65 U/L) (Table 1).

Serum creatine kinase level was significantly (P < 0.05) high in venom control group II mice (27.02±0.41 U/L), compared to Gr. I mice (9.55±2.50 U/L). HMBA treated Gr. III mice showed significant (P < 0.05) decrease in serum creatine kinase (18.96±0.40 U/L) as compared to Gr. II mice. Treatment with GNP-HMBA caused significant (P < 0.05) decrease in serum creatine kinase (12.20±0.96 U/L) compared to

Table 1— Effect of GNP-HMBA and HMBA on serum parameters in RVV induced nephrotoxic, myotoxic and hepatotoxic male albino mice

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Serum Creatinine (mg/dL)</th>
<th>Serum Urea (mg/dL)</th>
<th>Serum CK (U/L)</th>
<th>Serum LDH (U/L)</th>
<th>Serum GPT (U/L)</th>
<th>Serum GOT (U/L)</th>
<th>Serum ACP (U/L)</th>
<th>Serum ALP (U/L)</th>
<th>Serum γ-GT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr.1</td>
<td>0.26±0.01</td>
<td>24.4±1.75</td>
<td>9.55±2.50</td>
<td>156.19±10.00</td>
<td>26.96±0.4</td>
<td>18.46±0.43</td>
<td>1.07±0.08</td>
<td>110.78±21.11</td>
<td>2.31±0.07*</td>
</tr>
<tr>
<td>Gr.2</td>
<td>0.91±0.01*</td>
<td>49.70±1.37</td>
<td>156.19±10.00</td>
<td>26.96±0.4</td>
<td>18.46±0.43</td>
<td>1.07±0.08</td>
<td>110.78±21.11</td>
<td>2.31±0.07*</td>
<td></td>
</tr>
<tr>
<td>Gr.3</td>
<td>0.36±0.01*</td>
<td>44.55±0.44</td>
<td>156.19±10.00</td>
<td>26.96±0.4</td>
<td>18.46±0.43</td>
<td>1.07±0.08</td>
<td>110.78±21.11</td>
<td>2.31±0.07*</td>
<td></td>
</tr>
<tr>
<td>Gr.4</td>
<td>0.17±0.02*</td>
<td>39.00±0.23</td>
<td>156.19±10.00</td>
<td>26.96±0.4</td>
<td>18.46±0.43</td>
<td>1.07±0.08</td>
<td>110.78±21.11</td>
<td>2.31±0.07*</td>
<td></td>
</tr>
</tbody>
</table>

[Values were expressed as mean ± standard error of mean, statistical analysis was done with one way ANOVA, Gr.1, sham control, Gr.2 venom control, Gr.3 HMBA treated, Gr.4 GNP-HMBA treated. *P <0.05 when compared to venom control group with sham control group, #P <0.05 when compared to venom control group with HMBA treated group and GNP-HMBA treated]
Gr. II animals. GNP-HMBA gave more protection than HMBA against RVV induced myotoxicity in male albino mice (Table 1).

Neutralization of RVV induced hepatotoxicity by GNP-HMBA

RVV (10 µg/20 g/s.c.) significantly ($P < 0.05$) increased serum GPT, GOT, ACP, ALP and $\gamma$-GT level (49.65±0.35, 70.84±0.55, 5.24±0.18, 184.45±23.06 and 11.68±0.89 U/L, respectively) in venom control (Gr. II) mice as compared to the sham control (Gr. I) mice (26.96±0.4, 18.46±0.43, 1.07±0.08, 110.78±21.11 and 2.31±0.07 U/L, respectively), which indicated RVV had induced hepatotoxicity. Treatment with GNP-HMBA significantly ($P < 0.05$) decreased serum GPT, GOT, ACP, ALP and $\gamma$-GT level (28.19±0.61, 22.94±0.84, 2.50±0.10, 152.61±68.40 and 3.44±0.12 U/L, respectively). Protection against RVV induced hepatotoxicity offered by GNP-HMBA was more than that of HMBA (Table 1).

Acute induction of RVV caused significant changes in liver pathophysiology including loss of structural integrity, cellular infiltration, necrotic lesions, hemorrhage and dilated central vein. Treatment with GNP-HMBA showed partial recovery (no hemorrhage, less necrotic lesions, less cellular infiltration) of liver tissue. Treatment with HMBA did not show protection against RVV induced hepatocellular changes (Fig. 7).

Discussion

Snake bite is still an important socio-medical problem of the tropical and subtropical countries. Antisnake venom serum (ASVS) is the only available treatment but having many adverse effects and limitations. There is no specific dose of ASVS to neutralize specific snake venom. Search for alternatives of ASVS is an emerging interest of toxinologists. Several herbal antagonists identified in the past few decades failed to become an alternate of ASVS. The failure of the herbs accounts for many reasons among which geographical variation, availability, toxicity and efficacy are important. In some cases, pure compounds isolated from the herbs showed less protection than the whole extract. According to Ayurveda, metal conjugation with herbs improves the bioactivity of the herbs. Studies showed that the processing of metals in Ayurvedic formulations convert them into nanosized particles. Conjugation of nanoparticle with herb or herbal compounds may enhance the efficacy of the herbs or herbal compounds. Evidences have shown that gold nanoparticles conjugated with herbal compounds are effective drug delivery vehicles and have better cellular uptake.

In our recent study, root extract of *Vitex negundo*, an antisnake venom herb was used to synthesize gold nanoparticle, its physico-chemical characterization was done, and also evaluated for its *Naja kouthia* venom-neutralizing potential in animal model.

In the present study, hydrodynamic diameter of GNP and GNP-HMBA were 30-40 nm and 65-75nm, respectively. The difference of hydrodynamic diameter was due to capping of HMBA on the surface of gold nanoparticles. Zeta potential measures the stability of colloidal particles. In solution, gold nanoparticles scatter as colloids and its charge determined the zeta potential. A high positive or a high negative zeta potential induces repulsion force between the particles, which makes the particles more stable. In the present study, zeta potential of GNP-HMBA indicated moderate stability of the particles. Spectra analysis of FTIR confirmed the conjugation of HMBA with gold nanoparticle. TEM image showed the morphology and size of nanoparticles, which indicated the monodispersed nanoparticles formation. There was a difference between hydrodynamic diameter and TEM diameter of GNP-HMBA, which was due to the salvation layer on the surface of nanoparticles.

Russell's viper, one of the most dangerous snakes found in Asia, is responsible for the majority of snakebite incidents in India, Sri Lanka and Burma.
Nephrotoxicity is one of the major causes of death following Russell’s viper bite. Apart from kidney, RVV affects other organs also. Reported data showed that 18% Russell's viper bite caused nephrotoxicity, 14% caused myotoxicity and 1.2% caused hepatotoxicity and other organ toxicity. In the present study, induction of RVV caused an increase in serum creatinine, urea and urinary calcium, phosphate levels. Nephrons in kidneys are associated with filtration, reabsorption and secretion. Histopathology of kidney tissue showed congested glomerulus, cellular infiltration, inflamed and enlarged renal tubules with presence of blood, which hampered the normal nephron functions and the levels of creatinine, urea, calcium and phosphate were increased. Disturbances in kidney were indicated by high levels of serum creatinine. Increase in the kidney parameters was similar as found in previous study. High concentration of calcium and phosphorus in urine might be due to increased renal excretion indicating acute renal failure. Fibrin deposited in the renal tubules causing tubular blockade, thereby decreased renal filtration. As a result, the kidneys were unable to remove the increased levels of urea and creatinine from serum. Treatment with ASVS could not protect venom-induced nephrotoxicity which resulted in the development of acute renal failure. In the present study, treatment with GNP-HMBA significantly antagonized RVV induced renal changes (serum creatinine, urea and urinary calcium and phosphorous levels) probably through increased urinary filtration and reduced reabsorption.

Induction of RVV in experimental mice caused an increase in serum creatine kinase and lactate dehydrogenase due to myotoxic action of viper venom. Report showed that ASVS treatment is unable to neutralize venom induced myotoxicity. In the present study, GNP-HMBA significantly decreased serum creatine kinase level in RVV-induced male albino mice. Treatment with HMBA also neutralized RVV induced myotoxicity but degree of neutralization induced by GNP-HMBA was more than HMBA, which indicated increased efficacy of GNP-HMBA against viper venom induced myotoxicity. GNP-HMBA probably accumulated at the target sites (muscles), thereby increasing the clearance of creatine kinase and lactate dehydrogenase.

Transaminases (GOT, GPT, and $\gamma$-GT) and phosphatases (ACP and ALP) are normally present in hepatocytes, which may appear in circulation following the damage in hepatocyte plasma membrane. RVV-induced pro-oxidant formation has already been reported from the present laboratory which may indicate the hepatocyte membrane damage, resulting in increased transaminase and phosphatase levels in serum. In the present study, RVV induction in Gr. II animals caused an increase in transaminases and phosphatases. GNP-HMBA showed significant protection against RVV-induced hepatotoxicity in animal models. The probable cause of higher degree of hepatocyto-cellular protection induced by GNP-HMBA might be due to gold nanoparticle conjugation resulting in increased targeting at organ site, increased clearance and increased bio-detoxification. Histopathological study confirmed the loss of structural integrity, cellular infiltration, necrotic lesions, hemorrhage and dilated central vein in liver tissue induced by RVV and was partially recovered the histopathological changes indicating cellular targeting and damage repairing by GNP-HMBA.

In the present study, it has been observed that GNP-HMBA significantly gave protection against RVV, which was more than that of HMBA. The protection is likely due to the conjugation of HMBA with gold nanoparticle. Conjugation increased the accessibility of HMBA to the target sites, increased cellular uptake and repaired the damages induced by RVV. GNP-HMBA antagonized (directly/indirectly) the action of RVV-induced organ toxicity. The mechanism of action of GNP-HMBA should be explored in detail (bio distribution, bioavailability, bio targeting, bio detoxification, etc.) so as to encourage application of such herbo-nanoparticles against snake bite.

**Conclusion**

The present study indicated that GNP-HMBA significantly prevented RVV induced nephrotoxicity, myotoxicity and hepatotoxicity in male albino mice. It may be concluded that nanoparticles conjugation with herbal compound may open a new strategy in designing supportive therapy of ASVS treatment against snake venom in the near future.

**Conflict of interest**

The authors declare no conflict of interest.

**Acknowledgement**

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