Purification and characterization of a glycoprotein inhibitor of toxic phospholipase from *Withania somnifera*

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Abstract

A phospholipase inhibitor (WSG) has been purified from *Withania somnifera* using gel-filtration and ion-exchange chromatographies. The WSG is an acidic glycoprotein. Its molecular mass as determined by SDS–PAGE was 27 kDa. It neutralized the enzyme activity and pharmacological properties such as cytotoxicity, edema, and myotoxicity of a multi-toxic Indian cobra venom phospholipase (NNXia–PLA) but failed to neutralize the neurotoxicity. The glycan part of the molecule does not appear to be involved in any of the pharmacological properties studied. The results suggest that the neutralization of the pharmacological effects of the toxic phospholipase is brought about by inhibition of the enzyme activity by formation of a complex between the WSG and the toxic phospholipase. We report the purification and characterization of a glycoprotein phospholipase A inhibitor from *Withania somnifera*, medicinal plant.

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Keywords: *Withania somnifera*; Phospholipase A inhibitor; Antimyotoxic; Glycoprotein; *Naja naja* venom

There has been a tremendous growth in the search for alternative therapies and the therapeutic use of natural products, especially those derived from plants [1,2]. Plant sources have provided inspiration in the development of an impressive number of synthetic drugs. These resources provide a host of novel chemical compounds to yield therapeutic agents, which have been optimized on the basis of their biological activities. The most effective and accepted therapy for snakebite patients is immediate administration of specific or polyvalent antivenom following envenomation. Unfortunately variation in venom composition from one region to another renders it ineffective [3]. Antivenoms also carry an associated risk of anaphylaxis and serum reactions [4,5] and may not always prevent the local effects of envenomation such as myonecrosis, hemorrhage, and edema [6]. The use of plant extracts as antidotes for snake venom not only is common in places where they do not have prompt access to serum therapy but also is used as an adjunct to antivenin therapy. A number of plants with antidotal properties have been reported [7,8]. The plant isolates have become an attractive research material as an alternative for antiserum. A number of crude extracts and purified compounds have been reported to be active against snakebite [7,9,10]. The extracts are mixtures of several active compounds that neutralize the toxic properties of the venom. Such studies are not useful in understanding the mode of action of active compounds. The active compounds structurally resemble the secondary metabolites and this similarity is the basis for their physiological action. The activity may be attributed to the presence of enzymatic inhibitors, chemical inactivators, or immunomodulators present in the plant isolates [11]. Guerranti et al. [12] demonstrated that the presence of proteins from seed extract of *Mucuna pruriens* protected mice against the toxic effects of *Echis carinatus* venom. It does so by an immunological mechanism based on a series of specific epitopes common to some vegetal and venom proteins. Generally, the active compounds are multifunctional and possess more than one biochemical/pharmacological property. Interaction of such compounds with the toxins/enzymes leads to the neutralization/inhibition of their activities.
Lombardo and Dennis [13] demonstrated that Manoalide, a nonsteroidal sesterterpenoid isolated from sponge, inhibits cobra venom phospholipase activity.

Snake venoms are complex mixtures of proteins and peptides. Venoms of elapids mainly constitute α-neurotoxins, cardiotoxins and phospholipases which are the main factors involved in the toxic pharmacology and lethality of the venom [14]. Phospholipases, besides playing a digestive role in phospholipid hydrolysis, exert a wide variety of pharmacological activities such as neurotoxicity, cardiotoxicity, anticoagulant effect, inhibition of platelet aggregation, cytotoxicity, and edema-inducing activity [15–17].

*Withania somnifera* (Ashwaganda) is ethanobotanically reputed to be an antiulcer, antihepatotoxic, anti-inflammatory, antitumor, immunomodulator and is reported to be active against scorpion bite [18–21].

Earlier studies have shown that aqueous extracts of *W. somnifera* inhibit the phospholipase activity of *Naja naja* venom and increase the survival time of mice injected with the lethal dose. The present investigation reports isolation and characterization of an active glycoprotein (WSG)1 from *W. somnifera*, which neutralized the pharmacological effects induced by the basic phospholipase for the first time.

### Materials and methods

*Withania somnifera* roots were purchased from Panasari, Shivampet, Mysore, India. The roots were authenticated at the herbarium in the department of taxonomy at the Ayurveda Medical College, Mysore, India. Indian cobra (*N. naja*) venom (pooled and lyophilized from four to six adult snakes of both the sexes) was purchased from Hindustan Park (Kolkota, West Bengal). The NNXIa-PLA was purified by a combination of ion-exchange and gel-filtration chromatographies (data not shown). Male Swiss Wistar mice weighing 20–22g obtained from the Central Animal House Facility (Department of Zoology, University of Mysore, Mysore, India) were used for the pharmacological studies. The animal care and handling were conducted in compliance with the National Regulation for Animal Research. The animal experiments were carried out after review of the protocols by the Animal Ethical Committee of the University of Mysore.

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1 Abbreviations used: PLA, phospholipase A; WSG, *Withania somnifera* glycoprotein; BSA, bovine serum albumin; TFA, trifluoroacetic acid; PAS, periodic acid Schiff’s stain; ODS, octadecyl sulfate; EAT, Ehrlich ascites tumor; PBS, phosphate-buffered saline; CVV myotoxin, *Crotalus viridis viridis* myotoxin; ACL myotoxin, *Akebia contortrix lasticintus* myotoxin; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; GLC, gas–liquid chromatography; PLIs, phospholipase inhibitors.
determined by the phenol–sulfuric acid method [23]. Glucose (0–25 µg) was used as reference standard.

Determination of amino sugars. Amino sugars in WSG were determined by the colorimetric method of Ludoweig and Benmaman [24]. Glucosamine hydrochloride (0–50 µg) was used as reference standard.

Estimation of phenolic compounds. Phenolic compounds in WSG were determined by the colorimetric method as described by Swain and Hillis [25]. Phenol (0–40 µg) was used as reference standard.

PAGE and SDS–PAGE of WSG. PAGE was carried out for crude extract, WSI, WD3, and WSG on 10% polyacrylamide gel using Tris–glycine buffer (pH 6.8) according to the method of Davis [26]. The gels were stained with Coomassie brilliant blue R-250. SDS–PAGE (12.5%) was carried out according to the method of Laemmli [27] under reduced conditions. Crude extract, WSI, WSG, and low-molecular-weight markers (Sigma Chemicals) were loaded onto the gel. After electrophoresis the gel was stained with Coomassie brilliant blue R-250.

Determination of the molecular mass of WSG was also carried out by gel filtration according to the method of Andrews [28].

Detection of glycoprotein by periodic acid Schiffs (PAS) staining

SDS–PAGE was carried out in slab gels as described above. PAS staining was done according to the method of Leach et al. [29].

Gas–liquid chromatography (GLC). The WSG, (4 mg) in 1 ml of trifluoroacetic acid (2 N) was taken in a sealed tube. Hydrolysis was carried out at 100°C for 6–8 h in an oven. Acid in the hydrolysate was removed by flash evaporation in a water bath at 40°C and codistilled with water (1 ml × 3). The hydrolyzed sample was passed through Dowex 1/50 ion-exchangers to remove the amino acids. The neutralized and deionized sample was concentrated to 0.5 ml and subjected to preparation of alditol acetate derivative by the method of Sawardekar et al. [30]. A Shimadzu GLC (Model CR4 A) fitted with a flame ionization detector was used for analysis. OV-225 was the column used with a column injector and detector block. The temperature was maintained at 200, 225, and 250°C. Nitrogen at a flow rate of 40 ml/min was used as the carrier gas. The GLC–MS analysis of the sample was carried out as described by Jansson et al. [31]. The analysis was carried out on a Shimadzu GLC (Model QP 5000) using an SP 2380 capillary column. A temperature gradient of 180–200°C with an increase of 4°C/min was maintained for analysis, ionization potential was 70 eV, and mass range (m/z) was 40–400. Helium was the carrier gas used.

Interaction of WSG with NN–XIa–PLA. The incubated mixtures of NNXIa–PLA with WSG (1:1, w/w) and NNXIa–PLA and WSG were separately loaded onto a Sephadex G-50 column (1 × 50 cm) prequilled with PBS. Two-milliliter fractions were collected at a flow rate of 20 ml/h. Fractions were screened in a spectrophotometer at 280 nm.

Cytotoxicity determination. Cytotoxicity was determined using Ehrlich ascites tumor (EAT) cells grown in...
the peritoneal cavity of Swiss albino mice as described by Chwetzoff et al. [32]. EAT cells were suspended in Tyrode’s Ringer buffer (5 × 10^6 cells/ml) and incubated with NNXIa–PLA alone and NNXIa–PLA/WSG (0.5–2.0 M/M) for 30 min. Trypan blue saline (1%, 100 μl) solution was then added, and unstained (viable) cells were counted using a hemocytometer. The percentage of viable cells was determined by comparing the number of viable cells in control as 100%.

**Edema-inducing activity.** Edema-inducing activity was assayed according to the method of Yamakawa et al. [33]. NNXIa–PLA was incubated with various molar concentrations of the glycoprotein WSG (0.5–2 M/M) in 20 μl of saline for 1 h at 37°C. The incubated mixture was injected into the right footpad of the hind limb of mice (in groups of four to six) and 20 μl of saline in the left footpad of the hind limb. Control experiments were performed by injecting NNXIa–PLA alone. The increase in weight due to edema was calculated as the edema ratio, which equals the weight of edematous leg ×100/weight of normal leg.

**Myotoxicity determination.** NNXIa–PLA (half LD50 dose) in 0.3 ml of PBS was incubated with WSG (1:2 M/M) for 1 h at 37°C prior to injection (i.m) of the mixture into groups of five mice. After 2 h, the mice were anesthetized with diethyl ether. The abdominal cavity was opened and blood was collected from the abdominal vena cava. Creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) levels were determined in the serum by using the Span diagnostic kit. Control experiments were performed by injecting saline solution containing the WSG alone under similar conditions. Activity was expressed as IU/liter. The mice were incepted witheither NNXIa–PLA alone under similar conditions. The mice were injected i.m into groupsof five mice. After 2 h, the mice were anesthetized with diethyl ether. The percentage composition of tyrode’s Ringer buffer (5 × 10^6 cells/ml) and incubated with NNXIa–PLA/WSG (0.5–2.0 M/M) for 30 min. Trypan blue saline (1%, 100 μl) solution was then added, and unstained (viable) cells were counted using a hemocytometer. The percentage of viable cells was determined by comparing the number of viable cells in control as 100%.

**Characterization of WSG**

WSG showed a single band, whereas the crude extract and WSI showed a number of bands on PAGE (Fig. 3A). Similarly on subjecting crude extract, WSI, WSG, and protein molecular weight markers to SDS–PAGE under reduced condition, WSI gave a single band whereas WSI and crude extract gave several bands (Fig. 3B). The molecular weight of WSG was found to be 22.5 kDa by gel filtration and 27 kDa by SDS–PAGE. The WSG gave a single positive pink colored band on PAS staining (Fig. 2, inset). The carbohydrate contents of crude extract, WSI, and WSG are given in Table 1. WSG is composed of 23% carbohydrate, the protein–carbohydrate ratio being 1:0.3. Amino sugars contributed to 33% of the total carbohydrate in WSG (Table 1). The sugar composition of WSG revealed substantial amounts of glucose, galactose, and mannose along with other sugars like arabinose, xylose, rhamnose, and fucose. The percentage composition of each sugar calculated from the GC–MS profile is given in Table 2. Phenolic compounds were absent in WSG but were found in crude extract and in WSI (Table 1).

**Gel-permeation chromatography on Sephadex G-50 column**

The NNXIa–PLA, the WSG, and the interacted NNXIa–PLA with WSG in PBS (1:1, w/w) eluted at different elution volumes. Each of the them eluted as a single discrete peak. The NNXIa–PLA eluted at an elution volume of 38 ml, WSG at 34 ml, and the interacted sample at 20 ml. The elution of the interacted sample occurred before the elution of the individual components (Fig. 4).

**Antitoxic properties of WSG**

WSG completely neutralized the PLA activity of NNXIa at a ratio of 1:2 (M/M). NNXIa–PLA exhibited...
cytotoxicity on EAT cells. WSG inhibited the cytotoxicity in a dose-dependent manner and complete neutralization was observed at a concentration of 1:2 (M/M) (Fig. 5A).

The edema-inducing activity of NNXIa–PLA was effectively inhibited by WSG fraction in a dose dependent manner (Fig. 5B). Complete neutralization was observed at a concentration of 1:2 M/M.

The myotoxicity was confirmed by measuring plasma CPK and LDH activities that resulted in drastic increases of the activities at 2 h in comparison with control mice receiving PBS alone. WSG neutralized the myotoxicity induced by NNXIa–PLA completely at a molar ratio of 1:2 (PLA:WSG) as evidenced by the normal CPK and LDH values observed in the experimental animals (Fig. 5C). The result is confirmed by histopathological studies, which exhibited complete neutralization of myonecrosis with release of only a few inflammatory cells (Fig. 6).

Fig. 2. Reverse-phase HPLC analysis of WSG. WSG (20 μg) was applied on to a Shim-Pak (ODS) C18 (10 μm, 0.46 × 25 cm) column equilibrated with 0.1% TEA in water. Separation was achieved by a linear gradient of (0–100%) acetonitrile in 0.1% TEA. The flow rate was 1 ml/min and the elution profile was monitored at 280 nm. (Inset) PAS staining.

Fig. 3. (A) PAGE of crude extract (1), WSI (2), WD3 (3), and WSG (4) were loaded on 10% polyacrylamide gel and run at pH (8.8) using Tris–glycine buffer. The gel was run at 20 mA for 3 h. (B) SDS–PAGE pattern of crude (1), WSI (2), WSG (3), and low-molecular-weight markers (M) under reduced conditions on 12.5% gel. Molecular weight markers from top to bottom are in kilodaltons: ovalbumin (45), carboainhydrase (29), trypsin inhibitor (20), α-lactalbumin (14.2), and aprotinin (6.5).
Table 1
Composition of extracts and WSG

<table>
<thead>
<tr>
<th></th>
<th>Total Protein (mg)</th>
<th>Total carbohydrate (mg)</th>
<th>Protein:carbohydrate</th>
<th>Amino sugars (mg)</th>
<th>Phenols (mg)</th>
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<tr>
<td>Crude</td>
<td>180</td>
<td>25</td>
<td>1:0.14</td>
<td>8.6</td>
<td>56</td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>WSI</td>
<td>32</td>
<td>6</td>
<td>1:0.19</td>
<td>1.2</td>
<td>Nil</td>
</tr>
<tr>
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<td>16</td>
<td>1:0.14</td>
<td>3.3</td>
<td>48</td>
</tr>
<tr>
<td>WD3</td>
<td>6.3</td>
<td>1.69</td>
<td>1:0.19</td>
<td>0.68</td>
<td>Nil</td>
</tr>
<tr>
<td>WSG</td>
<td>2.1</td>
<td>0.840</td>
<td>1:0.3</td>
<td>0.28</td>
<td>Nil</td>
</tr>
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</table>

Table 2
Composition of sugar as determined by GC–MS of WSG

<table>
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<tr>
<th>Sugars (%)</th>
<th>Glu</th>
<th>Gal</th>
<th>Man</th>
<th>Xyl</th>
<th>Ara</th>
<th>Rham</th>
<th>Fuc</th>
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<tbody>
<tr>
<td>WSG</td>
<td>25</td>
<td>19.2</td>
<td>16.3</td>
<td>12.2</td>
<td>12.2</td>
<td>8.1</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Fig. 4. Interaction of NNXIa–PLA and WSG. Gel filtration of (▲) NNXIa–PLA (W) WSG and (●) preincubated NNXIa–PLA and WSG on a Sephadex G-50 column. The column (1 × 50 cm) was preequilibrated with phosphate-buffered saline (PBS) 0.1 M, pH 7.4. The samples were loaded separately and eluted with the same equilibrating buffer. Fractions of 2 ml were collected at a flow rate of 20 ml/h at room temperature. Protein elution was monitored at 280 nm.

Fig. 5. Neutralization of pharmacological effects of NNXIa–PLA by WSG. Effect of increasing molar concentration of WSG on (A) cytotoxicity, (B) edema-inducing activity, and (C) myotoxicity (■, CPK activity; □, LDH activity) of NNXIa–PLA. Values represent means ± SE (n = 5). *P < 0.01, when compared to NNXIa–PLA activity.
Modification studies

Modification of the histidine residue of NNXIa–PLA with p-bromophenacyl bromide resulted in the loss of catalytic, cytotoxic, edema, and myotoxic activities.

Discussion

A number of plant extracts have been reported to possess detoxifying effects on snake venoms [3,8,11]. Though there are a number of reports on plants from different geographical areas, which neutralize the toxicity of snake venom, only a few attribute such activity to the identified chemical compounds. Wedelolactone was shown to exert several well-defined pharmacological activities such as antinociceptive, antihemorrhagic and antiproteolytic [36–38]. Among the purified components stigmasterol, sitosterol, flavonoids, glycosides, caffeic acid, and coumarins are common constituents of plants like Eclipta prostrata, Alpinia speciosa, and Schumannophyton magnificum which exhibit anti-snake-venom activity [7]. The action of the extracts on neutralization of snake venom is attributed to the presence of more than one active compound. The identified active substances are mostly low-molecular-weight compounds that exhibit more than one biological or pharmacological property in addition to an antidotal effect. To mention a few, caffeic acid, curcumin, flavonoids, and their derivatives possess anti-inflammatory, hepatoprotective, and anticarcinogenic activities. Mors et al. [7] has observed a striking parallelism between the capability of plants and their chemical components, which are anti-inflammatory and antihepatoxic as well as neutralizing the snake venom toxicity. The extracts of W. somnifera are reported to contain Withanolides, Withanolidine glycosides, C-28 steroid lactones, saccharose, β-sitosterol, and macromolecules [39–41]. Most of the compounds noted above have structural similarities to compounds mentioned earlier and also possess anti-inflammatory, antihepatoxic, and immunomodulatory activities [18–21]. These compounds derived from plants structurally resemble secondary metabolites, which is the basis for their physiological action [10]. Despite identifying several active compounds from plant isolates only a few studies explain the mechanism of action of the compound. With the aid of circular dichroism studies Vishwanath et al. [42] found that aristolochic acid forms a 1:1 complex with PLA2, acting like a noncompetitive inhibitor of the toxic PLA2. Tsai et al. [43] demonstrated the association between aristolochic acid and PLA2 that resulted in significant change in the secondary structure of the enzyme. Wedelolactone inhibits myotoxicity of CVV myotoxin from Crotalus viridis viridis, bothropsin from Bothrops jararacus, and ACL myotoxin from Akistrodon contortrix laticinctus venoms by binding to toxins. Studies carried out by Melo and Ownby [38] have demonstrated that polyanions like chondroitin sulfate and heparin bring about the neutralization of the myotoxicity of Bothrops venom partly by nonspecific acid–base complex formation and partly due to more specific interaction with the proteins.

The macromolecular fraction from W. somnifera root extract inhibited the phospholipase activity of the Indian cobra N. naja venom and also increased the survival time of mice injected with the lethal dose of the venom. In contrast the micromolecular fraction enhanced the PLA activity.

Characterization of glycoprotein WSG

WSG, a glycoprotein, was isolated from the roots of W. somnifera by subjecting it to buffer extraction followed by gel-filtration and ion-exchange chromatography. A number of phenolic compounds are known to be associated with plant extract, for example, hydroxy benzoic acid, flavonoids, tannins, and coumarins [7,10,44]. The phenol and phenolic derivatives were excluded during the purification step and hence the glycoprotein was free of phenols. Molecular mass determined by the two methods varied 22.5 kDa by gel filtration and 27 kDa by SDS–PAGE. We have used the higher molecular mass for all calculations. The composition of sugars by GC–MS showed larger proportions

Fig. 6. Light micrograph of longitudinal sections of thigh muscle of mice (40×) after im injection: (A) control, (B) NNXIa–PLA, and (C) NNXIa PLA and WSG at 1:2 M/M ratio. Widespread necrotic cells (M), inflammatory cells (I), and disorganized nuclei are observed in (B).
of hexoses (61%) and lower proportions of pentoses, which comprised the remaining (39%) (Table 2). A fluorescence emission maximum was found to be at 342 nm, indicating the presence of exposed tryptophan residues in the molecule (data not shown).

Several glycoprotein inhibitors of snake venom PLA₂’s have been isolated from the blood plasma of venomous and nonvenomous snakes [45–48]. They are invariably acidic α-globulins that inactivate PLA₂ by forming soluble complexes. In addition to their anti-PLA₂ properties, some of these factors also neutralize the toxic effects induced by a particular PLA₂. CgMIP-I and CgMIP-II purified from blood plasma inhibited the myotoxicity of the two variants enzymatically active (myotoxin I) and inactive, Lys-49 (myotoxin II), isolated from Cerophidian godmani [49]. Six phospholipase inhibitors (PLIs) isolated from a range of Australian elapid sera, were able to protect in vivo the lethal effects of homologous PLA₂ [50]. The PLIs are generally composed of two protein chains, an α-chain and a β-chain. The α-chains are 20- to 30-kDa glycoprotein subunits and the β-chains are nonglycosylated 20- to 25-kDa protein subunits. The carbohydrate moiety is found not to effect the in vitro function of the inhibitor [50,51]. The WSG is also an acidic glycoprotein similar to the α-chain of the PLIs but in contrast it is composed of a single protein. Carbohydrate moieties did not contribute to the inhibitory activity of the catalytic and the myotoxic effects of the NNXIa–PLA.

Melo and Ownby [38] used gel filtration to demonstrate the complex formation between heparin and a myotoxic PLA from B. jararacussu venom. This is evident by the increased molecular mass of 37 kDa (equal to 1:1 complex between glycoprotein and NNXIa–PLA) observed by gel filtration (Fig. 4). The displaced movement of the associated protein may be considered as proof of association. The association appears to be due to electrostatic interactions between the negatively charged groups of acidic glycoprotein and the positive charge of basic phospholipase. The interacted complex could not be cited on the electrophoresis. However, such an electrostatic association of glycoprotein WSG was not found with a basic PLA₂ NNXIII and VRV-PL-VIIIa from N. naja and Vipera ruselii venoms, respectively (data not shown). Therefore, the electrostatic interaction of the glycoprotein and PLA₂ may also require specific basic residues in a specific conformation site.

Neurotoxic NNXIa–PLA is toxic to EAT cells and also induces edema, and myotoxicity in mice. WSG neutralized both cytotoxicity and edema induced by NNXIa–PLA₂ in a dose-dependent manner. One of the mechanisms by which PLA₂’s induced edema is by release of lysophosphatides and fatty acids [52]. However, enzyme activity is not strictly required to induce this effect [53]. Caseria sylvestris is known to inhibit the edema induced by B. jararaca [54]. Elaeodendron balae root extract, used to cure swelling, is also effective against snakebite toxicity [8].

Venoms from snakes of several different families cause myonecrosis. Homma and Tu [55] divided the muscle cell necrosis produced by 37 venoms into three types based on histological features. These groups were designated as (1) coagulation type, (2) myolysis type, and (3) mixed type, which consists of both of the types in equal numbers. Generally, elapid venoms exhibit the second type of myonecrosis due to the presence of either myotoxic phospholipases or cardiotoxins in the venoms. Ownby and Colberg [56] noted that the N. naja venom-induced myonecrosis observed was similar to that induced by the phospholipase myotoxins such as notexin, taipoxin, crototoxin, and Bothrops species myotoxins [57–61]. Numerous specific toxic phospholipase A₂’s have been isolated from elapid venoms like Pseudechis australis venom. PLA₂’s Pa-IG, Pa-5, Pa-12, and Pa-15 are characterized as being homologous single-chain postnaptically acting basic PLA₂’s (with the exception of Pa-IG which is uniquely acidic) that damage muscle fibers resulting in loss of muscle contractility [62]. The myotoxicity induced by NNXIa–PLA is the myolysis type. Histopathological studies support the myolysis type myotoxicity. The WSG completely neutralized the myonecrotic activity of the NNXIa–PLA supported by the significant inhibition of serum LDH and CPK value of the NNXIa–PLA and the integrated muscle. Myonecrosis following snake envenomation is due to the presence of phospholipase myotoxins, hence the neutralization may be achieved by anti-enzyme action to inhibit the myotoxic PLA₂ in venom [7,38]. Fukami and Hattori [63] reported that persimmon tannin inhibited completely the necrotic lesions induced by habu, mamushi, and cobra venom. Crude extract of Eclipta prostrata and its three active compounds wedelolactone, sitosterol and stigmasterol showed antagonism against myotoxic effects of Bothrops venom [8,38]. WSG neutralized the cytotoxicity, edema, and the myotoxic effects induced by NNXIa–PLA. WSG inhibited the PLA activity at a molar ratio 1:2 (Data not shown) as well as neutralized the pharmacological effects of the PLA at the same molar ratio. The catalytic histidine-modified NNXIa–PLA was devoid of cytotoxicity, edema inducing activity and myotoxic effects. Therefore all the three pharmacological effects appear to depend on the PLA activity of the toxin. However, the neurotoxic effect of the PLA is not neutralized by WSG, while histidine-modified PLA failed to induce any neurotoxic effects. Modification of active site histidine by p-bromophenacyl bromide converts a neurotoxic PLA into non neurotoxic protein. WSG fails to transform a neurotoxic PLA into a non-neurotoxic PLA due to its weak electrostatic interaction with the toxin.

WSG a glycoprotein similar to α-proteins of snake serum is purified and characterized from W. somnifera. Interaction of WSG with multitoxic N. naja PLA
suggests that WSG appears to confer beneficial effects against snake venom toxicity. Further antivenom properties of this molecule are being investigated.

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