Proteins from *Mucuna pruriens* and Enzymes from *Echis carinatus* Venom

**CHARACTERIZATION AND CROSS-REACTIONS***

*Mucuna pruriens* seeds have been widely used against snakebite in traditional medicine. The antivenin property of a water extract of seeds was assessed in vivo in mice. The serum of mice treated with extract was tested for its immunological properties. Two proteins of *Echis carinatus* venom with apparent molecular masses of 25 and 16 kDa were detected by Western blot analysis. These results demonstrated that the observed antivenin activity has an immune mechanism. Antibodies of mice treated with non-lethal doses of venom reacted against some proteins of *M. pruriens* extract. Proteins of *E. carinatus* venom and *M. pruriens* extract have at least one epitope in common as confirmed by immunodiffusion assay.

Snakebite is a considerable problem in certain tropical and subtropical countries. According to World Health Organization estimates, 40,000 of 5 million cases of snakebite are fatal. Antivenins obtained from horses treated with snake venom are one of the principal remedies against snakebite. This therapy has the disadvantage that antivenins must be given immediately, and snakebite victims may develop an adverse reaction including anaphylactic shock (1). The use of endogenous plants with a reputation against snakebite is therefore worth considering (2).

In preliminary experiments (3, 4) we demonstrated that extract of *M. pruriens* (MPE),1 a medicinal plant widely used in Nigeria for its chemical and pharmacological properties, protects mice against the lethal effect of *Echis carinatus* venom (EV). Both MPE and EV are heterogeneous mixtures, their interaction represents a complex phenomenon, and there is no information about its biochemical mechanism. EV contains proteins with different toxic properties including opposite effects on blood clotting. Well known proteins are: disintegrins EC3 (5), EC6 (6), and echistatin (7), which inhibit the interaction of fibrinogen with the glycoprotein IIb-IIIa receptor on the platelet surface; echicetin (8) and ECLVIXXhp (9) with an opposite effect on platelet aggregation; two metalloproteases, earcin (10, 11) and carinactivase (12), which are prothrombin activators and act as procoagulant enzymes; and phospholipase A2 (PLA2) (13–15), the most abundant enzyme, which has many effects including inhibition of prothrombin activation by earcin and carinactivase (16). When injected into mice, this complex mixture of proteins induces disseminated intravascular coagulation leading to death in less than a day. The composition of the *M. pruriens* seed is also complex and variable, with 20–30% protein (lectins, globulins, protease inhibitors), 1–10% fat, 4–5% ash, 4–9% water, 4–7% fiber (17–20), and L-DOPA (21), an interesting non-protein component. The aim of the present study was to study the mechanism, the factors of MPE, and the proteins of EV involved in the observed phenomenon.

**EXPERIMENTAL PROCEDURES**

**Materials**—Crude venom of *E. carinatus* (common name: saw-scaled viper; family: Viperidae; subfamily: Viperinae; genus: *Echis*; species: *E. carinatus*) was purchased from Sigma. Authenticity was certified by Miami Serpentarium Laboratories for its quality and representation of species. PBS, ammonium persulfate, Temed, dithiothreitol, and peroxidase-conjugated goat anti-mouse IgG were from Sigma. IgG from Humana Milchunion eG. LMW marker, PlusOne silver staining kit protein, HiPrep 26/10, HiTrap protein G column, Sephacryl S-200 HR, agarose, and ECL (enhanced chemiluminescence) detection kit were from Amersham Biosciences. Nitrocellulose membrane, 30% acrylamide/bis solution, and Tris/glycine SDS buffer were from Bio-Rad.

**Plant Material and Animals**—*M. pruriens* (family: Fabaceae; subfamily: Papilionoideae; genus: *Mucuna*; species: *pruriens*) seeds were collected in the Rukuba area in Jos, Nigeria with the aid of a traditional healer. They were authenticated by Prof. S. W. H. Hussini of the Department of Botany, University of Jos. Voucher specimen number A102 is deposited in the Pharmacy Herbarium of the University of Jos.

*Preparation and Partial Purification of *M. pruriens* Seed Extract*—Sundried seeds of *M. pruriens* were ground to a paste of uniform consistency, 50 g of which was soaked in 100 ml of H$_2$O, extracted for 24 h at 4 °C, and centrifuged at 10,000 × g for 20 min. The supernatant lyophilized to a powder (24% protein), which was stored at −4 °C. Separation of the protein (P) and non-protein (NP) fractions was

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1 The abbreviation used are: MPE, *Mucuna pruriens* extract; PLA$_2$, phospholipase A$_2$; EV, *Echis carinatus* venom; PBS, phosphate-buffered saline; P, protein fraction of *M. pruriens* extract; NP, non-protein fraction of *M. pruriens* extract; LC/ESI-MS/MS, liquid chromatography/ electrospray ionization-tandem mass spectrometry; CTL, C-type lectin domain; CRD, carbohydrate-recognition domain; anti-MPE, serum of mice treated with MPE; anti-P, serum of mice treated with P; anti-P2, serum of mice treated with P2; anti-P3, serum of mice treated with P3; anti-EV, serum of mice treated with EV; EV25, 25-kDa EV protein; EV16, 16-kDa EV protein; MPE 22–28, 22–28-kDa *Mucuna pruriens* proteins; Temed, N,N,N,N'-tetramethylethylenediamine.

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‡ The abbreviations used are: MPE, *Mucuna pruriens* extract; PLA$_2$, phospholipase A$_2$; EV, *Echis carinatus* venom; PBS, phosphate-buffered saline; P, protein fraction of *M. pruriens* extract; NP, non-protein fraction of *M. pruriens* extract; LC/ESI-MS/MS, liquid chromatography/electrospray ionization-tandem mass spectrometry; CTL, C-type lectin domain; CRD, carbohydrate-recognition domain; anti-MPE, serum of mice treated with MPE; anti-P, serum of mice treated with P; anti-P2, serum of mice treated with P2; anti-P3, serum of mice treated with P3; anti-EV, serum of mice treated with EV; EV25, 25-kDa EV protein; EV16, 16-kDa EV protein; MPE 22–28, 22–28-kDa *Mucuna pruriens* proteins; Temed, N,N,N,N'-tetramethylethylenediamine.
achieved by gel filtration on a HiPrep 26/10 column: 5 ml of MPE solution (5.4 mg/ml) was loaded and eluted with 50 mM Tris buffer, pH 7, at a flow rate of 5 ml/min. The fractions were monitored at 280 nm and for their conductivity.  

\[ E_{280\ nm}, \ E_{254\ nm}, \ \text{and conductivity were monitored.} \]

For partial purification of MPE proteins, 5 ml of MPE solution (5.4 mg/ml) was applied to a Sephacryl S-200 HR column (2.6 x 80 cm) eluted with 50 mM Na₂HPO₄, pH 7.5, at a flow rate of 1.5 ml/min and read at 280 nm. P₁, P₂, and P₃ were obtained.

**In Vivo Protective Effect of MPE, P, and NP against EV**—Groups of eight mice were injected with MPE, P₁, P₂, P₃, or saline (control). At different times after injection, the mice were injected with a minimum lethal dose of EV (minimal lethal dose, 2 mg/kg). The percentage of survivors was assessed 24 h later. The control group was injected with saline before EV. All fractions were injected intraperitoneally at doses proportional to body weight, calculating dilution after separation.

**Preparation of Antisera and Purification of IgG**—Six groups of eight mice were treated once a week for 3 weeks with MPE, P₁, P₂, P₃, EV (non-lethal dose), and saline. After 28 days they were sacrificed, blood was withdrawn, and anti-MPE, anti-P₁, anti-P₂, anti-P₃, anti-EV, and preimmune sera were obtained. All antisera were purified by affinity chromatography with an AKTA liquid chromatography system. 10 ml of each anti-serum diluted in binding buffer (20 mM Na₂HPO₄, pH 7) and filtered on a 0.22-μm membrane was adsorbed on a 5-ml protein G column (1.6 x 2.5 cm) equilibrated in binding buffer until all unbound material was washed out. IgG fractions were then eluted at a flow rate of 2.5 ml/min with 0.1M glycine-HCl, pH 2.7 (elution buffer). The fractions were neutralized with 1 M Tris, pH 9, and concentrated with Centriplus membrane (final concentration of 1.5 mg/ml).

**SDS-PAGE and Western Blot Analysis**—Proteins in all fresh samples were determined by a Bio-Rad assay (22) and separated by 12% SDS-PAGE according to Laemmli (23). They were transferred to a nitrocellulose membrane (0.45 μm) at 100 V for 1 h at 4 °C and stained with Ponceau S. The membrane was blocked with 0.3% non-fat powdered milk in 1× PBS containing 0.1% Tween 20 and incubated overnight at 37 °C with treated mouse IgG. IgG of untreated mice was used as the negative control. The membrane was washed in 1× PBS containing 0.1% Tween 20, incubated with peroxidase-conjugated goat anti-mouse IgG (1:2000), and developed by ECL. Detection of total proteins after 12% SDS-PAGE was achieved by silver staining using PlusOne™ silver staining kit protein.

**Neutralization of Lethal Potency of EV**—The minimal lethal dose of EV was preincubated with 100 μl each of anti-MPE, anti-EV, anti-P₁, anti-P₂, anti-P₃, and preimmune IgG fractions at 37 °C for 1 h. The preincubated mixtures were then injected into six groups of eight mice.

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**Figures:**

**Fig. 1. Chromatographic separation of P and NP fractions of MPE.** 5 ml of MPE solution (5.4 mg/ml) was loaded on a HiPrep 26/10 column and eluted with 50 mM Tris buffer, pH 7, at a flow rate of 5 ml/min. The fractions were monitored at 280 and 254 nm and for their conductivity.  

**Fig. 2. Chromatographic purification of MPE by gel filtration.** A. 5 ml of MPE solution (5.4 mg/ml) was applied to a Sephacryl S-200 HR column (2.6 x 80 cm) and eluted with 50 mM Na₂HPO₄, pH 7.5, at a flow rate of 1.5 ml/min. Three protein peaks at 280 nm (P₁, P₂, P₃) and a non-protein fraction (NP) were obtained. B, SDS-PAGE of MPE and the fractions obtained in A were loaded and silver-stained. Molecular masses of LMW marker are indicated on the left (St). AUFS, absorbance units at full scale.
Control groups were injected with EV mixed with saline or purified IgG from serum of preimmune mice. The number of deaths in the subsequent 24 h was recorded.

**Immunodiffusion Assay**—Antigenic relationships between the various antigens were studied by double diffusion test, according to Ouchterlony and Nilsson (24). Holes 5 mm in diameter were punched in

*Fig. 3.* Immunological detection of EV and MPE proteins. A, Western blot analysis of EV with anti-MPE IgG: MPEr, 2 μg of MPE proteins under reducing conditions (positive control); EVr, 90, 60, and 30 μg of venom proteins under reducing conditions; and EVnr, 90, 60, and 30 μg of venom proteins under non-reducing conditions. Anti-MPE IgG was diluted 1:200 in blocking buffer and incubated overnight at 37 °C. EV, silver staining of venom proteins under reducing conditions. Molecular masses of LMW marker are on the right. B, Western blot analysis of EV with anti-P1, anti-P2, and anti-P3 IgG. 1 μg of P1, P2, and P3 were positive controls for their respective IgG. For EVr, 60 μg and 120 μg of venom proteins were dissolved in reducing buffer and loaded in lanes 1 and 2, respectively. Molecular masses of standard are on the left. Anti-P1, anti-P2, and anti-P3 IgG were diluted 1:200 in blocking buffer and incubated overnight at 37 °C. C, Western blot analysis of MPE proteins with anti-EV IgG; EVnr, 2 μg of venom proteins under non-reducing conditions (positive control); MPEnr, 3, 9, and 27 μg of Mucuna proteins under non-reducing conditions; MPEr, 3, 9, and 27 μg of Mucuna proteins under reducing conditions. Anti-EV IgG was diluted 1:400 in blocking buffer and incubated overnight at 37 °C. MPEr (right) shows silver stain of reduced MPE proteins. For each experiment, binding was detected by incubating the membrane with peroxidase-conjugated goat anti-mouse IgG, diluted 1:2000 in blocking buffer. Proteins were then visualized by ECL at a short exposure time.
horizonal gels containing 1% agarose in 1× PBS. Protein fractions of MPE or EV (20 μl) were placed in the peripheral wells and IgG in the central well. Diffusion was allowed to proceed for 24 h at 37 °C. The gel was then washed with saline and dried. The precipitin line was visualized with Coomassie Brilliant Blue.

Enzymatic In-gel Digestion—Coomassie Blue-stained MPE and EV protein bands separated by SDS-PAGE were excised from the gel and digested with trypsin according to known procedures (25) with slight modifications. Briefly, gel slices were washed for at least 1 h in 100 mM NH₄HCO₃, pH 8.0, and then for 1 h with 50% acetonitrile,100 mM NH₄HCO₃, pH 8.0, under shaking. Acetonitrile was added to shrink the gel pieces, and after 10–15 min of incubation, the solvent was removed and the samples were dried in a Speed Vac. Gel slices were reswollen with 25 mM NH₄HCO₃, pH 8.0, containing modified trypsin (Promega) and incubated for 4 h at 37 °C. The supernatant was acidified with trifluoroacetic acid to a final concentration of 1%. Peptides were extracted from the gel slices twice with 60% acetonitrile and 0.1% trifluoroacetic acid for 20 min. All supernatants were combined, and after evaporation to near dryness, peptide fragments were reconstituted in 20 μl of 0.1% trifluoroacetic acid.

Liquid Chromatography/Electrospray Ionization-Tandem Mass Spectrometry (LC/ESI-MS/MS) Analyses—The mixture of peptides was separated using a Nucleosil C8 column (4.6 × 250 mm, 5-μm particle size, 300 A) and analyzed with a Finnigan LCQ ion trap mass spectrometer (San Jose, CA) with an ESI source. A detailed scheme of the experimental setup for this type of analyses is described elsewhere (26). Briefly, a positive voltage of 3 kV was applied to the electrospray needle, and a N₂ sheath flow was applied to stabilize the ESI signal. The LC/MS analysis was conducted using a PerkinElmer high pressure liquid chromatography system coupled to the LCQ. The mobile phase from the column (flow rate of 0.5 ml/min) was split before the mass detector by a Tee-connector. The enzymatically digested peptides were eluted from the column using 0.5% formic acid in water (mobile phase A) and 0.5% formic acid in acetonitrile (mobile phase B) with a three-step linear gradient of 5–10% B in the first 10 min, 10–35% B in the next 40 min, and 35–40% B in the last 5 min. The LC/ESI-MS/MS analysis was accomplished using an automated data acquisition procedure in which a cyclic series of three different scan modes was performed. Data acquisition was conducted using the full scan mode (m/z 300–2000) to obtain the most intense peak (signal > 1.5 × 10⁵ counts) as the precursor ion, followed by a high resolution zoom scan mode to determine the charge state of the precursor ion and MS/MS scan mode to determine the structural fragment ions of the precursor ion. The resulting MS/MS spectra were then matched against a protein data base (Owl) by Sequest software to confirm the sequence of tryptic peptides.

RESULTS

Antivenin Activity of MPE—We first found that the protective effect of MPE against the lethal effect of EV was exerted at a dose of 21 μg/g and was evident 24 h and 1–4 weeks after administration. To understand the chemical nature of substances responsible for the protection, an in vivo test was set up with two fractions obtained by HiPrep separation of MPE, one containing P and the other one NP compounds from MPE (Fig. 1). NP fractions contain small molecules like L-DOPA responsible for E₂₅₀ nm absorbance, free amino acids, ions, and fatty acids as already reported (18, 19).

The in vivo test showed that P and NP fractions exerted protection in different ways. As shown in Table I, the NP fraction conferred short term protection (1 day), whereas the P fraction conferred long term protection (1, 2, and 3 weeks after administration). When we comparatively injected mice with MPE, P, and NP fractions once a week for 3 weeks, the protective effect of the P fraction specifically increased. Some compounds in the NP fraction may be adjuvants in the long term protective effect of the P fraction was less active when used alone. Only with a booster dose of P fraction was the total effect restored.

Three well resolved protein peaks, P1, P2, P3, and NP fraction were obtained by further purification of MPE by gel filtration on Sephacryl S-200 HR. They are shown in Fig. 2.

Antibodies against EV Induced in Mice by Extracts of M. pruriens—The protective activities of MPE, P1, P2, and P3 were tested for their capacities to raise antibodies in mice against EV proteins. IgG were purified from anti-MPE, anti-P1, anti-P2, anti-P3, and preimmune mice serum with protein G affinity separation and used in Western blot experiments.

When anti-MPE IgG were tested against EV proteins, two protein bands with apparent molecular masses of about 25 kDa (EV25) and 16 kDa (EV16) were detected. The signal was only visible under reducing conditions implying that the epitope on the native EV protein was in a cryptic state. When MPE proteins were incubated with the specific anti-MPE IgG, as positive control, a pattern similar to that obtained with silver staining was achieved, indicating that almost all MPE proteins were highly antigenic (Fig. 3A).

When the protein fractions obtained by partial purification of MPE on Sephacryl S-200 were injected into mice, we also obtained antibodies against EV. Antibodies raised in mice by injecting P2 fraction gave similar results to those obtained with anti-MPE IgG, whereas when anti-P3 IgG was used, only EV16 was detected. No signal against any EV proteins was obtained using IgG of mice treated with P1 fraction (anti-P1 IgG). Positive controls of anti-P1, -P2, and -P3 IgG were the corresponding P1, P2, and P3 fractions (Fig. 3B).

Antibodies against Proteins of M. pruriens Induced in Mice by Administration of EV—Under reducing conditions, some anti-EV IgG reacted with at least three bands of MPE proteins with molecular masses in the range of 22–28 kDa (MPE 22–28) as shown in Fig. 3C. These proteins mainly belong to the P2 fraction. EV proteins reacted strongly against the specific anti-EV IgG under non-reducing conditions, and this sample was used as positive control.

Neutralization of Lethal Potency of EV—The capacity of anti-MPE, anti-P1, anti-P2, and anti-P3 IgG to neutralize the toxicity in vivo of EV was tested after incubation of the fractions with venom, and results are reported in Table II. No neutralization was observed in control groups 1 and 2 (negative controls); lethal potency of EV was neutralized by anti-EV IgG obtained from mice treated with a non-lethal dose of EV (group 3, positive control). Anti-MPE (group 4) and anti-P2 (group 6) showed neutralizing effects similar to that of group 3; less neutralization was observed with anti-P3 (group 7), and no neutralizing effect was obtained with anti-P1 (group 5). A 50% survival percentage was considered a satisfactory neutralizing effect.

Immunodiffusion Test—To confirm the results of Western blot experiments and to ascertain the presence of one or more common antigenic epitopes in MPE and EV proteins, the double diffusion test was used. When anti-MPE IgG was tested.
FIG. 5. Identification of EV16 band by mass spectrometry. The most abundant peaks of the tryptic digested mixture of EV16 band were selected, tandem mass spectrum was performed, and the sequence stretch, together with its starting mass, its end mass, and the molecular weight of the peptide were entered in the data base search program (Sequest) where they were converted to a peptide sequence tag. Two peptide were partially sequenced. A, NLQFAEMIVK corresponds to the fragmentation of ion with m/z 670.5. B, DNLNTYDKK corresponds to the fragmentation of a ion with m/z 982.2.

| Seq | # |   b         |   y         | (+1) |
|-----|---|-------------|-------------|------|
| N   | 1 | 115.1       |             | 11   |
| L   | 2 | 228.3       | 1226.5      | 10   |
| F   | 3 | 375.4       | 1113.3      | 9    |
| Q   | 4 | 503.6       | 966.2       | 8    |
| F   | 5 | 650.7       | 838.0       | 7    |
| A   | 6 | 721.8       | 690.9       | 6    |
| E   | 7 | 850.9       | 619.8       | 5    |
| M   | 8 | 982.1       | 490.7       | 4    |
| I   | 9 | 1095.3      | 359.5       | 3    |
| V   | 10| 1194.4      | 246.3       | 2    |
| K   | 11| -           | 147.2       | 1    |

| Seq | # |   b         |   y         | (+2) |
|-----|---|-------------|-------------|------|
| N   | 1 | 58.1        |             | 11   |
| L   | 2 | 114.6       | 613.8       | 10   |
| F   | 3 | 188.2       | 557.2       | 9    |
| Q   | 4 | 252.3       | 483.6       | 8    |
| F   | 5 | 325.9       | 419.5       | 7    |
| A   | 6 | 361.4       | 345.9       | 6    |
| E   | 7 | 426.0       | 310.4       | 5    |
| M   | 8 | 491.6       | 245.8       | 4    |
| I   | 9 | 548.2       | 180.2       | 3    |
| V   | 10| 597.7       | 123.7       | 2    |
| K   | 11| -           | 74.1        | 1    |

| Seq | # |   b         |   y         | (+1) |
|-----|---|-------------|-------------|------|
| D   | 1 | 116.1       |             | 9    |
| N   | 2 | 230.2       | 996.1       | 8    |
| L   | 3 | 343.4       | 882.0       | 7    |
| N   | 4 | 457.5       | 768.8       | 6    |
| T   | 5 | 558.6       | 654.7       | 5    |
| Y   | 6 | 721.7       | 553.6       | 4    |
| D   | 7 | 836.8       | 390.4       | 3    |
| K   | 8 | 965.0       | 275.3       | 2    |
| K   | 9 | -           | 147.2       | 1    |

| Seq | # |   b         |   y         | (+2) |
|-----|---|-------------|-------------|------|
| D   | 1 | 58.5        |             | 9    |
| N   | 2 | 115.6       | 498.5       | 8    |
| L   | 3 | 172.2       | 441.5       | 7    |
| N   | 4 | 229.2       | 384.9       | 6    |
| T   | 5 | 279.8       | 327.9       | 5    |
| Y   | 6 | 361.4       | 277.3       | 4    |
| D   | 7 | 418.9       | 195.7       | 3    |
| K   | 8 | 483.0       | 138.2       | 2    |
| K   | 9 | -           | 74.1        | 1    |
against MPE and EV proteins, a pattern shown in Fig. 4a was obtained indicating coalescence of antigens. When anti-EV IgG was tested against MPE, P2, P3, and EV one precipitin line was obtained indicating coalescence of antigens. When anti-EV IgG was preincubated with the venom was neutralized when the venom was preincubated with saline and then EV. Survivors were counted 24 h after EV injection. All fractions were injected intraperitoneally with doses proportional to body weight (µg/g). All groups received one injection except group A in the last column, which was immunized with one injection a week for 3 weeks.

**DISCUSSION**

The present results demonstrate that extracts of *M. pruriens* seeds protect mice against the toxic effects of EV. It does so by an immunological mechanism based on a series of specific epitopes common to some vegetal and venom proteins.

The *in vivo* test results indicate that administration of MPE proteins raised antibodies responsible for the protection observed. The long term protection was in fact more complete when the P fraction was administered according to an immunization protocol (once a week for 3 weeks). Several proteins in the P2 fraction were involved in raising antibodies, while the most purified P3 fraction is less active. We demonstrated by Western blot analysis that certain antibodies induced in mice by injection of vegetal extracts reacted directly with certain EV proteins, and immunodiffusion experiments pointed out the cross-reaction between MPE and EV proteins.

The present results demonstrate that extracts of *M. pruriens* seeds protect mice against the toxic effects of EV. It does so by an immunological mechanism based on a series of specific epitopes common to some vegetal and venom proteins.

**TABLE I**

| Fractions | Dose | Survivors/Total |
|-----------|------|-----------------|
| MPE       | 21 µg/g | 4/2             |
| P         | 21 µg/g | 4/2             |
| NP        | 21 µg/g | 0/8             |
| Control   | 0 µg/g  | 0/8             |

**TABLE II**

Neutralization activity of IgG fractions

Minimal lethal dose of EV was incubated at 37°C for 1 h with 100 µl of the IgG fractions indicated (mixture). This was the dose for a 30-g mouse. Five groups of 12 mice were used. Groups 1 and 2 were negative controls, group 3 positive control.

| Group | Mixture | Survivors/Total |
|-------|---------|-----------------|
| 1     | EV + saline | 0/12           |
| 2     | EV + preimmune IgG | 0/12          |
| 3     | EV + anti-EV IgG | 7/12           |
| 4     | EV + anti-MPE IgG | 6/12           |
| 5     | EV + anti-P2 IgG | 0/12           |
| 6     | EV + anti-P2 IgG | 5/12           |
| 7     | EV + anti-P3 IgG | 3/12           |
Snakebite and other coagulation disorders. They may contain procoagulant and anticoagulant activities, very likely they could interfere in the coagulation process. Several plant proteins, such as echicetin, ECLVIX/XBp, and carinactivase, which show procoagulant and anticoagulant activities, may contain the same CTL domain of calcium-dependent type lectin-related proteins of EV.

We can conclude that when MPE proteins are injected into mice in such a way as to induce an antibody production, a polyclonal serum against epitopes present on one or more EV proteins is obtained. If MPE extract and some of its proteins protect mice against EV PLA2 or other snake venom proteins that show procoagulant and anticoagulant activities, very likely they could interfere in the coagulation process.

The present findings open new perspectives in the field of vaccine by natural products and may be useful in the therapy of snakebite and other coagulation disorders.

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