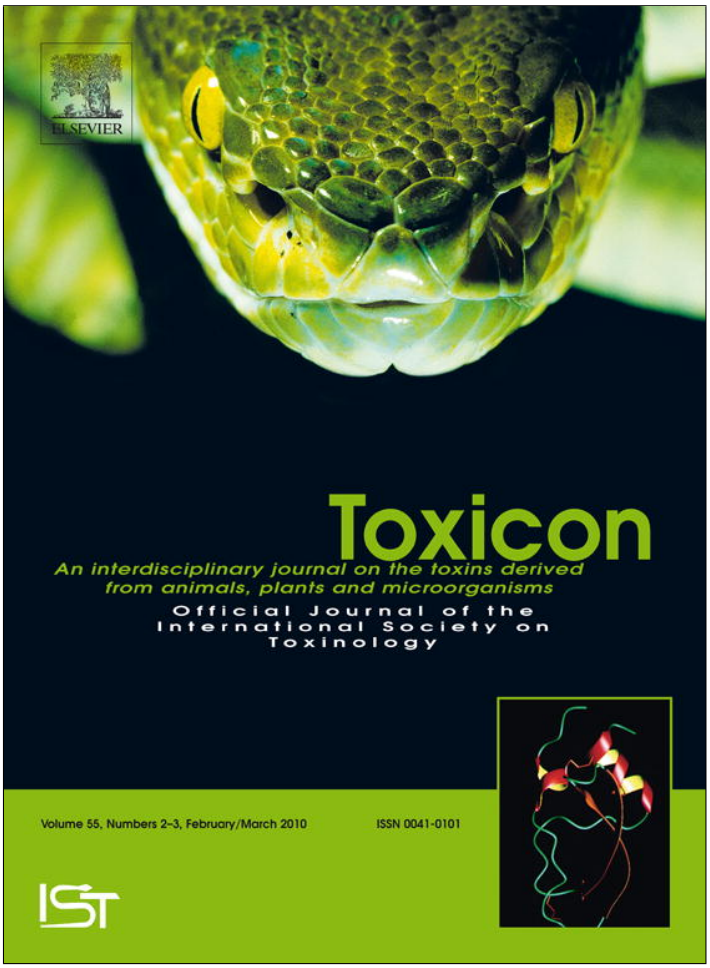

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Neurotoxic and cytotoxic effects of venom from different populations of the Egyptian *Scorpio maurus palmatus*

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ABSTRACT

Neurotoxic and cytotoxic effects of venoms from *Scorpio maurus palmatus* taken from different populations were assessed for geographic based variability in toxicity, and to evaluate their insecticidal potency. Scorpions were collected from four regions. Three locations were mutually isolated pockets in the arid area of Southern Sinai. The fourth sample was collected from a population inhabiting the semi-arid environment of Western Mediterranean Coastal Desert. The neurotoxic (paralytic) effect of the venom from each population was assayed by its ability to induce permanent disability in adult cockroaches within 3 h. Venom was applied using microinjection techniques through an intersegmental membrane. Probit analysis was used to calculate the Paralytic Effective Dose (PED_{50}, ng/100 mg). Levels of glutathione, lipid peroxidation, protein carbonyl content and nitric oxide, as well as the activities of superoxide dismutase, catalase and cholinesterase, were measured to assess the cytotoxicity of the venom. The results show that the injected venom from each population induced obvious spasticity, followed by flaccid paralysis. All the tested biochemical parameters, except glutathione content, revealed significant differences in toxicity in venom taken from the different scorpion populations. We conclude that (i) the venom of this scorpion has significant neurotoxic and cytotoxic effects on insect cells, (ii) its efficacy, as assessed by the PED_{50} unit, exhibited variation across its geographic range, and (iii) components in the venom may have the potential for being developed into effective and environmentally friendly bioinsecticides.

1. Introduction

Within the phylum of Arthropoda, scorpions (Chelicerata, Scorpionida) are the oldest known terrestrial species. Scorpions are particularly well adapted to survival in extreme habitats and their ability to produce and deliver venoms is an important factor in this success (Newton et al., 2007). Scorpion venom is a complex mixture composed of a wide array of substances. It contains mucopolysaccharides, hyaluronidase, phopholipase, low molecular mass molecules like serotonin and histamine, protease inhibitors, histamine releasers and polypeptidyl compounds (Martin Eauclaire and Couraud, 1995; Valdez-Cruz et al., 2007; Feng et al., 2008; Kanoo and Deshpande, 2008). Scorpion venom peptides exhibit a vast variety of biochemical activities and pharmacological functions. They can be classified into disulfide-bridged and non-disulfide bridged peptides (Goudet et al., 2002; Zeng et al., 2005).

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Most scorpion toxins contain three to four disulfide-bridges. They can recognize and specifically interact with ion channels including Na^+, K^+, CI^- and Ca^{2+} (Rodriguez-de-la-Vega and Possani, 2004). Scorpions present an immense combinatorial peptide library for the potential development of insecticides and pharmaceuticals which to date has gone largely untapped.

Several scorpion-derived insecticidal toxins have been identified and have been classified into several groups. The first group can be labeled the contracture-inducing insect toxins or excitatory toxins (Zlotkin et al., 1971; Zlotkin et al., 1985) which cause immediate paralysis. These are single-chained polypeptides of 70 amino acids which bind to insect synaptosomal membranes (Darbon et al., 1982). Excitatory toxins induce spastic paralysis. This spasticity is caused by repetitive activity of motor nerves resulting from the activation of sodium currents at more negative membrane potentials (Pelhate and Zlotkin, 1982; Zlotkin et al., 1991; Froy et al., 1999). The total specificity of excitatory toxins to insects highlights them as targets for the design of potential bioinsecticides (Gurevitz et al., 2007). The second group of toxins are considered depressants (Zlotkin et al., 1985; Kopeyan et al., 1990; Strugatsky et al., 2005; Karbat et al., 2007). The depressant toxins are structurally similar to α-toxins but which are inactive in mammals. They induce a progressive and flaccid paralysis of blowfly larvae and cockroaches. Electrophysiological studies revealed that depressant toxins suppress evoked action potentials due to a strong depolarization of the axonal membrane (Ben Khalifa et al., 1997; Strugatsky et al., 2005). The final group of toxins are potent both on mammals and insects (Loret and Hammock, 2001; Gordon et al., 2003; Cohen et al., 2006). The recent demonstration that insect-specific natural toxins can be used to construct recombinant viral insecticides gives new incentive to apply scorpion toxins to another urgent problem: control of agricultural pest insects (Stewart et al., 1991; Gordon, 1997; Regev et al., 2003; Whetstone and Hammock, 2007).

The scorpion toxins composition and venom potency are different from species to species (Watt and Simard, 1984; Dyason et al., 2002; Borges et al., 2006). Differences have been also described within the same species concerning protein content and toxicity of the venoms (El Ayeb and Rochat, 1985; Martin et al., 1987; Kalapothakis and Chavez-Olortegui, 1997). These differences can be observed in different individual venoms collected from different specimen at the same time (Omran and McVean, 2000; Pimenta et al., 2003; Newton et al., 2007; Abdel-Rahman et al., 2009) and also in the venom of the same specimen following multiple extraction during time (Kalapothakis and Chavez-Olortegui, 1997; El-Hafny et al., 2002). *Scorpio maurus palmatus* (*S. m. palmatus*) belongs to the family Scorpionidae and is common in the Mediterranean, Middle East, Saudi Arabia and Jordon regions. It is found in the Coastal Plain of Libyan Desert, Lower Egypt, Southern and Central Sinai. Each scorpion lives alone in a burrow, but hundreds of burrows may be found in some areas (Levy and Amitai, 1980). *S. m. palmatus* have been chosen for this study because (i) it is common in Egypt and widely distributed in both arid (910–1676 m above sea level) and semi-arid habitats; (ii) it lives solitarily in burrows that can reach 100 cm or more in depth; and (iii) the unique composition of its venom as well as its pharmacological properties. Previously, we have found that scorpion venom of the Egyptian *S. m. palmatus* collected from different biotopes has different profiles (at both DNA and protein levels) and these differences are sex-dependent (Abdel-Rahman, 2008; Abdel-Rahman et al., 2009). The main goal of this communication is to examine and estimate the neurotoxic and cytotoxic effects of *S. m. palmatus* venom collected from different locations in Egypt and to assess whether the pharmacological properties of the venom taken from these different populations vary with geographic location, as well as to evaluate the insecticidal potency of this specific venom. To the best of our knowledge, this is the first study of its kind on this scorpion species.

2. Materials and methods

2.1. Collection of venom samples

Samples of *S. m. palmatus* were collected from two different geographical regions in Egypt, the Sinai Peninsula and the North coast. Scorpions were captured from three different locations (Wadi Sahab, El-Agramia and Rahaba Plains) in the southern region of the Sinai Peninsula (910–1676 m above sea level), an area geographically separated from the Western Mediterranean Coastal Desert (30.5 m above sea level) by the Suez Canal and Gulf of Suez from where a second group of scorpions were collected (Abdel-Rahman et al., 2009). Captive scorpions from the four locations were kept separately in individual containers. Scorpions were milked using the squeezing method according to Abdel-Rahman et al. (2009) and individual venom samples collected and lyophilized. The freeze-dried pooled venom was stored at −80 °C prior to use.

2.2. Test animals and toxicity assays

The cockroach *Periplaneta americana* was chosen as the experimental animal due to its proven sensitivity to scorpion neurotoxins (Fishman et al., 1997). Laboratory bred adult female cockroaches of 1 ± 0.1 g body weight were used throughout the study. The paralytic potency (neurotoxic effect) was assayed by the ability of scorpion venom to make adult cockroaches droop dorsally and have difficulty in returning to their normal stance within 3 h after venom application. Using microinjection technique, venom was applied (each dose was tested on 12 individuals) through the intersegmental membrane between the third and the fourth abdominal segments (to prevent excessive bleeding, a patch of melted parafilm was placed over the site of injection). Venom was dissolved in a physiological solution (0.15 M NaCl containing 1 mg/ml bovine serum albumin) and volumes of 0.5–2.0 μl per insect were applied. Probit analysis (Finney, 1971) was used to determine the median paralytic effective dose (PED_{50}, ng/100 mg body weight). This was the dose that induced paralysis in 50% of treated insects within 3 h of treatment.

2.3. Cytotoxic assays

The cytotoxic effects of crude venom from each location were tested 24 h after venom treatment. The adult cockroaches (12 insects per group) were injected with the same dose (119 ng/100 mg), and a control group was injected with physiological solution. The following biochemical parameters were measured in the insect tissues: the level of lipid peroxidation (LP), protein carbonyl contents (PCC), glutathione (reduced form) content (GSH), the level of nitric oxide (NO), and the activities of Cu/Zn-superoxide dismutase (Cu/Zn-SOD), catalase (CAT) and acetylcholinesterase (AChE).

2.3.1. Malondialehyde (MDA) assay

MDA formation was used to quantify the lipid peroxidation level in the insect tissues. It was measured using the thiobarbituric acid (TBA) assay according to the method of Uchiyama and Mihara (1978) by reacting of 0.5 ml of a 10% (w/v) insect homogenate with 1 ml of 6% thiobarbituric acid (Winlab, UK) and 3 ml of 1% phosphoric acid for 45 min in boiling water. The colour of TBA chromogen was measured at 520 and 532 nm (Helios α UNICAM UV-Visible Spectrophotometer, UNICAM, Cambridge, UK). The difference between absorbance at 520 nm and at 530 nm is used to determine the TBA value which represents the malonaldehyde concentration and was taken as the measure of lipid peroxidation in the insect tissues. The level of lipid peroxidation was expressed as micromoles MDA per milligram of tissue. The compound 1,1,3,3-tetramethoxypropane was used as an external standard.

2.3.2. Protein carbonyl contents (PCC)

Protein carbonyl content was measured by forming labeled protein hydrazone derivatives, using 2,4-dinitrophenylhydrazide (DNPH), which were quantified spectrophotometrically (Reznick and Packer, 1994). Insects were homogenized using a Teflon/glass homogenizer in phosphate-buffered saline (PBS) at 50 mg tissue/ml and centrifuged at 12.000 *g* to remove debris. After precipitation of protein with an equal volume of 1% trichloroacetic acid (TCA), the pellet was resuspended in 1 ml of DNPH 10 mM in 2 N HCl. Separate blanks were prepared by adding 1 ml of 2 N HCl without DNPH. Samples were left at room temperature for 1 h in the dark and vortexed every 15 min. An equal volume of 20% TCA was added and after centrifugation at 12,000 *g* for 15 min at 4 °C, pellets were washed three times with 1 ml of ethanol:ethylacetate mixture (1:1) to remove the free DNPH and lipid contaminants. The final pellet was dissolved in 1 ml of 6 M guanidine and kept at 37 °C for 1 h in a water bath with mixer. The solution was centrifuged for 15 min 12,000 g. The carbonyl content (nanomoles/mg) was calculated from peak absorption (370 nm) using an absorption coefficient (e) of 22,000 M^{-1} Cm^{-1}.

2.3.3. Glutathione reduced form (GSH)

The reduced form of glutathione (GSH) in control and the treated insects was estimated according to the method of Beutler et al. (1963). GSH levels were calculated using a standard curve prepared with known amounts of GSH (Aldrich Chemical Co., Germany).

2.3.4. Cu/Zn-superoxide dismutase (Cu/Zn-SOD) and catalase (CAT)

SOD assay was performed by the method of Misra and Firdovich (1972). The rate of inhibition of auto oxidation was monitored at 560 nm; the amount of enzyme required to produce 50% inhibition is defined as one unit of enzyme activity. The SOD activity was expressed as units/g. Catalase activity was assayed using the method of Aebi (1974). Catalase activity was determined by following the decomposition of H_2O_2 at 240 nm.

2.3.5. Estimation of nitric oxide (NO)

This assay is used for determination of nitrite as an indicator of nitric oxide production in biological samples. Nitric oxide is rapidly converted to nitrite and nitrate in typical oxygenated aqueous solutions. Nitrite, a stable end-product of nitric oxide, was measured in the supernatant by a spectrophotometric method based on the Griess reaction (Green et al., 1982). A solution of sodium nitrite was used as a standard and measured in the range of 0.25–50.0 μM.

2.3.6. Estimation of acetylcholinesterase activity (AChE)

The activity of acetylcholinesterase in both the treated and control groups was estimated by the spectrophotometric method described by Ellman et al. (1961) using kits obtained from Biomerieux Company (Marcy l'Etoile, France).

2.4. Statistical analysis

Data were statistically analyzed using SPSS software (Statistical Package for Social Science, Version 12.0). Descriptive analyses including mean and standard error were applied to all biochemical measurements in control and treated groups. The effect of scorpion venom on insect cells for each population was assessed using the Student's unpaired *t*-test (Snedecor, 1956) between control and treated groups. Group differences were considered statistically significant at the level of $P < 0.05$. One-way ANOVA was used to assess differences between the treated groups of the four scorpion populations in all the biochemical measurements. Pearson's Correlation was applied to determine the relationship between the oxidative stress biomarkers (LP and PCC) and the antioxidants (GSH, NO, SOD and CAT). Canonical Discriminant Function Analysis (DFA, a multivariate technique) was used (based on the biochemical data) to examine similarities and differences in toxicity tests between the four scorpion populations. It maximizes among-group distances while shrinking within-group dispersion to resolve patterns among groups (Albrecht, 1980).

3. Results

3.1. Neurotoxic effect

The median paralytic effective dose (PED_{50}) of the crude venom of *S. m. palmatus* from each location is presented in Fig. 1. The data shows that the internal application induced clear paralytic effects on the adult cockroaches over the time course of venom application (3 h). Also, the efficacy of

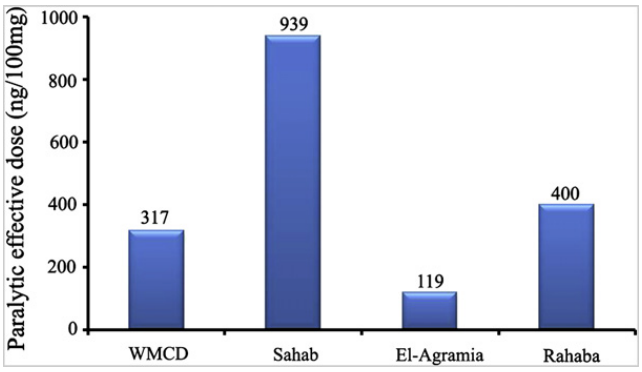

Fig. 1. Comparison between the paralytic effective dose (PED_{50} ng/100 mg/body mass) of the injected venom of the four scorpion populations (WMCD, Sahab, El-Agramia (Agramia), Rahaba) at 180 min post venom application.

S. m. palmatus venom, as determined by the PED_{50} unit, exhibited variation (from 119 to 939 ng/100 mg) between the four scorpion populations. Moreover, within the Sinai populations the venom of El-Agramia Plain was the most effective while the venom from the Wadi Sahab scorpions was the least effective. Venom from El-Agramia scorpions induced paralysis as early as 60 min post injection and recorded the lowest PED_{50} value (119 ng/100 mg) at 180 min compared with the other scorpion populations. In the treated groups from WMCD, Sahab and Rahaba intoxication started within 80 min post injection and peaked around 160 min. All groups of insects assayed with *S. m. palmatus* venom exhibited a primary excitatory phase in the form of fast walking followed by an obvious decrease in locomotion. Three hours after venom application, the insects drooped dorsally and had difficulty in returning to the normal standing position. They were unable to right themselves. The normal behavior pattern of the untreated insects considered as the control group which always active, walking and climbing on the glass containers, or sometimes touching each other.

3.2. Cytotoxic effect

To evaluate variability in cytotoxic effects of *S. m. palmatus* venom, the lowest PED_{50} (119 ng/100 mg) at 180 min of the crude venom from each location was injected into adult cockroaches. The oxidative stress biomarkers, non-enzymatic and enzymatic antioxidants as well as the activity of cholinesterase were measured 24 h post venom injection. The effect on the oxidative stress biomarkers (LP and PCC) is graphically presented in Fig. 2. Concentrations of the lipid peroxidation product (MDA) in the treated insects of both WMCD and El-Agramia were significantly decreased ($P < 0.05$) compared to the controls while the concentration of MDA non-significantly decreased in the Sahab and Rahaba-treated groups. One-way ANOVA between the four

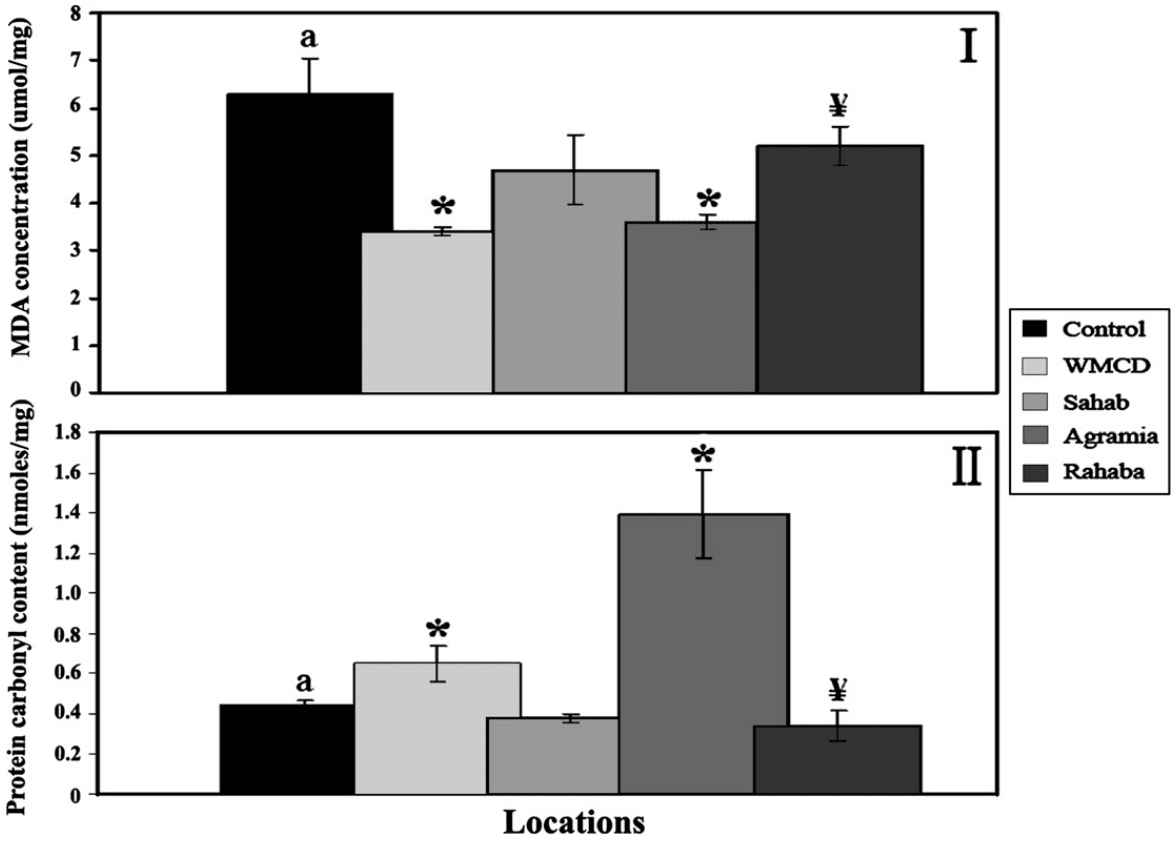

Fig. 2. Effect of injected *Scorpio maurus palmatus* venom (PED_{50}: 119 ng/100 mg) collected from the four different regions on the level of lipid peroxidation (I) and protein carbonyl content (II) of the adult cockroach *Periplanita americana* 24 h post treatment. [a], Values are presented as mean ± S.E. of 12 insects per group. * Significant difference between control and treated group using Student's unpaired *t*-test ($P < 0.05$). ¥ Significant difference between treated groups using one-way ANOVA ($P < 0.05$).

Table 1
Effect of injected *Scorpio maurus palmatus* venom (PED_{50}: 119 ng/100 mg) collected from the four different regions on the antioxidant measurements and acetylcholinesterase in the adult cockroach *Periplanita americana* 24 h post treatment.

Parameters	Control	Treated Groups			
		WMCD	Sahab	El-Agramia	Rahaba
GSH (μg/g)	911.0 ± 27.0[a]	911.0 ± 36.0	951.0 ± 56.0	1217.0 ± 173.0	949.0 ± 35.0
NO (μM/g)	14.0 ± 0.24	15.0 ± 0.3	15.0 ± 0.4	25.0 ± 3.0*	14.0 ± 0.4**
SOD (U/g)	77.0 ± 11.0	43.0 ± 14.0	75.0 ± 2.0	34.0 ± 11.0*	56.0 ± 13.0
CAT (U/g)	15.0 ± 0.80	13.0 ± 2.0	10.0 ± 1.0*	6.0 ± 1.0*	10.0 ± 1.0* & **
AChE (U/g)	255.0 ± 22	135.0 ± 15.0*	182.0 ± 7.0*	160.0 ± 7.0*	106.0 ± 12.0* & **

*Significant difference between control and treated group using Student's unpaired *t*-test ($P < 0.05$).
**Significant difference between treated groups using one-way ANOVA ($P < 0.05$).
[a] Values are presented as mean ± S.E. of 12 insects per group.

treated groups (WMCD, Sahab, El-Agramia and Rahaba) revealed a highly significant difference ($F_{1,3} = 10.45$, $P < 0.001$) in the concentration of lipid peroxidation product (Fig. 2I). Protein carbonyl concentrations in the cockroaches treated with WMCD and El-Agramia venom were substantially higher ($P < 0.05$) than the control group. PCA content was non-significantly decreased in the treated groups of Sahab and Rahaba. Highly significant differences in protein carbonyl concentrations was observed between the four treated groups using one-way ANOVA ($F_{1,3} = 8.63$, $P < 0.001$: Fig. 2II).

Table 1 illustrates the influence of injected scorpion venom (119 ng/100 mg) on the non-enzymatic antioxidants, GSH and NO, as well as the activities of Cu/Zn-SOD, CAT and AChE in the four treated groups. Although GSH showed some increase, especially in El-Agramia-treated group, the increase was not statistically significant. A similar pattern emerged for the NO assay but the increase was statistically significant in El-Agramia-treated group. One-way ANOVA showed a marginally significant difference in the level of NO of the treated groups, with $F_{1,3}$ value equals 3.1, $P < 0.05$. The pattern was similar for Cu/Zn-SOD (Table 1) with a significant decrease in the El-Agramia-treated group but no significant changes in the other three groups. There was no significant difference in the activity of SOD between the treated groups using one-way ANOVA ($F_{1,3} = 1.6$; $P < 0.22$). In contrast, the activity of CAT significantly decreased ($P < 0.05$) in the three groups from Southern Sinai. We detected a marginally significant difference ($F_{1,3} = 3.0$; $P < 0.05$) between the four treated groups using one-way ANOVA. The data in Table 1 shows that there was a prominent decrease ($P < 0.05$) in the activity of AChE in all four treated groups. One-way ANOVA revealed a highly significant difference in the activity of AChE between the four treated groups ($F_{1,3} = 7.7$; $P < 0.001$).

Discriminant Functions Analysis (DFA) was used to evaluate the variability in the physiological effects of injected venom (119 ng/100 mg) between sites (WMCD, Sahab, El-Agramia and Rahaba). Table 2 and Fig. 3 illustrate that the four treated groups were distinct along the first and second axes based on the biochemical measurements. Along the first axis (Function 1), which represents 64.5% of the discrimination, El-Agramia-treated group had positive values against negative ones for Sahab and Rahaba-treated groups, while WMCD-treated group was in between. The positive values of this axis were mainly correlated with the PCC and NO. Along the second axis (Function 2), which represents 22.2% of variation, the venoms from Southern Sinai scorpions had negative values against positive ones for WMCD-treated group. The negative values were correlated with AChE and SOD. The third axis (Function 3) separated El-Agramia venom from the other sources. It represented 13.3% of the discrimination. Also, it correlated with CAT, GSH and MDA.

4. Discussion

4.1. Neurotoxic and cytotoxic mode of action of S. m. palmatus venom

Scorpion venom is mainly directed at arthropods and, specifically, soft-bodied insects serving as prey food. Hence, the selective pressure in scorpion venom may lead to the selection of efficient insect toxins such as excitatory contractive toxins and flaccid depressant toxins (Gurevitz et al., 1998; Gordon et al., 2003). The results presented here showed that the crude venom of *S. m. palmatus* collected from different biotopes in Egypt induced paralytic effects on the adult cockroach *P. americana*. The paralytic efficiency of the crude venom of this species could be attributed to the presence of different neurotoxic fractions, slow lethal and fast reversibly paralytic factors (Lazarovici et al., 1982). A clear cooperative interaction was demonstrated between the slow lethal and fast reversibly paralytic factors, resulting in an evident of the original toxicity to insects of the crude venom. Lazarovici et al. (1982) concluded that the purified insect toxins IT_1 and IT_2 caused a reversible

Table 2
Structure matrix pooled within-group correlations between discriminating variables and standardized canonical discriminant functions.

Biochemical parameters	Function 1 (64.5%)	Function 2 (22.2%)	Function 3 (13.3%)
Protein carbonyl content (PCC)	0.683[a]	0.071	0.441
Nitric Oxide (NO)	0.440[a]	−0.216	−0.416
Acetylcholinesterase (AChE)	0.099	−0.351[a]	−0.209
Cu/Zn-SOD	−0.218	−0.279[a]	−0.222
Catalase (CAT)	−0.186	0.316	−0.501[a]
Glutathione reduced form (GSH)	0.352	0.237	0.431[a]
Lipid perioxidation (MDA)	−0.223	−0.205	0.225[a]

[a] The largest absolute correlation between each variable and any discriminant function.

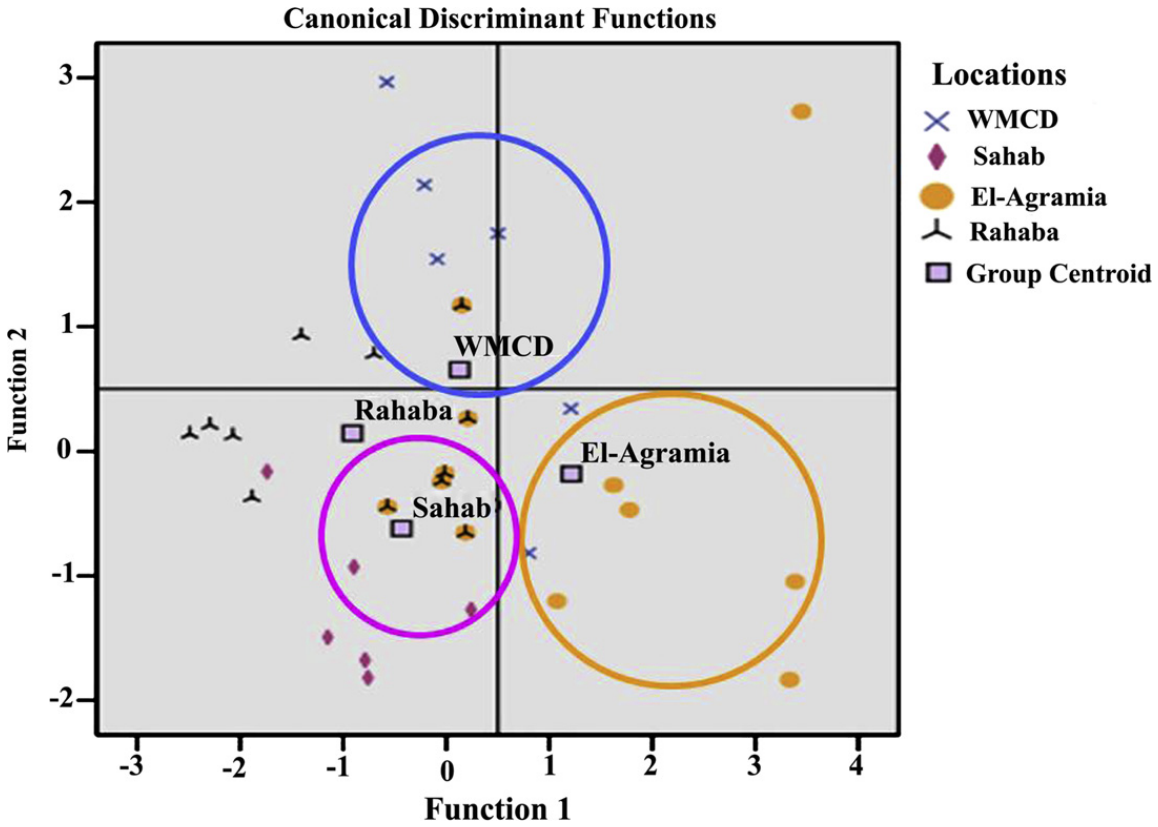

Fig. 3. Discriminant Functions Analysis (DFA) based on the biochemical measurements of the scorpion venom-treated groups of the four different locations (WMCD, Sahab, El-Agramia and Rahaba).

blockage of both the sodium and potassium currents when assayed on the isolated axon of the adult cockroach *P. americana* under current and voltage clamp conditions. This may explain the specific symptomatology and the mechanism of paralysis induced by these toxins to an insect.

In this communication, we observed that all the treated insects showed uncontrolled hyper-excitability followed by paralysis. These symptoms may be due to the presence of two polypeptide toxins (maurocalcine and maurotoxin) in this specific venom. Maurocalcine (MCa) is a 33 amino acid residue peptide that was isolated from the venom of the scorpion *S. m. palmatus*. (Boisseau et al., 2006). MCa is a potent activator of the ryanodine receptor (RyR1), a calcium channel receptor responsible for the release of calcium from intracellular stores (Esteve et al., 2003; Pouvreau et al., 2006). In particular, increased plasma membrane permeability to Ca^{2+} and other ions results in ionic imbalances, followed by subsequent rupture of the sarcolemma that is coincident with cell death. Accumulation of large amounts of calcium in cells may alter the integrity and function of several membrane systems and affect mitochondrial energy production (Klaassen and Watkins, 1999).

On the other hand, maurotoxin (MTX: 34 amino acid residues) may play a crucial role in the paralytic effect of *S. m. palmatus* venom. MTX is a potent and selective inhibitor of the intermediate conductance subtype of calcium-activated potassium channels that lower the efficacy of excitatory stimuli (Castle et al., 2003). Also, MTX has been reported to display a variety of pharmacological activities such as blocking insect (Shaker B) voltage-gated Kv channels heterologously expressed in *Xenopus laevis* oocytes (Avdonin et al., 2000; Carlier et al., 2000). Furthermore, the paralytic potency of the crude venom of *S. m. palmatus* might be related to the inhibitory effect of the crude venom on the activity of AChE in all treated insects (Table 1). It is well known that AChE hydrolyzes acetylcholine to choline and acetic acid and terminates its' action at the postsynaptic membrane. By inhibiting acetylcholinesterase, the endogenous acetylcholine accumulates in the synaptic clefts and neuroeffector junctions. The excess acetylcholine in turn stimulates cholinoreceptors to evoke increased responses followed by muscle spasm and paralysis (Pappano, 2001), a condition called spastic paralysis. Ryan and Byrne (1988) reported that six terpenoids (derived from electric eel) inhibited the enzyme acetylcholinesterase and elicited the appropriate *in vivo* effects of insect paralysis and mortality.

Reactive oxygen species (ROS) are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function, or excessive quantities, the state called oxidative stress (Nordberg and Arner, 2001). The present study revealed that the level of lipid peroxidation (MDA) decreased significantly in the treated insects especially with WMCD and El-Agramia scorpion populations' venom (Fig. 2). The decrease in the MDA concentrations might be due to the reduction in metabolic rate of the treated insects (as a defense mechanism) to minimize the rate of oxygen

utilization. This is confirmed by the recorded severe and sustainable muscle spasm that might deplete oxygen and energy resources as well as increasing the oxygen debt. Previous studies indicated that the concentration of lipid peroxidation is affected by metabolic rate of the organisms (Misra and Gorsky, 1981; Sohal et al., 1981; Abdel-Rahman et al., 1999). Moreover, the decrease in lipid perioxidation level could be attributed to the significant increase of NO concentration in the treated group of El-Agramia (Table 1). NO (as a free radical scavenger) has been shown to inhibit lipid peroxidation in cell membranes (Hog and Kalyanaraman, 1998; Rubbo et al., 2000).

On the other hand, we observed substantial increase in the level of protein carbonyls in the treated insects of WMCD and El-Agramia (Fig. 2). Increased carbonyls in the treated groups could be associated with the damage in the skeletal muscles after venom applications. It was noticed that *S. m. palmatus* venom induced hyper-excitability and severe muscle cramps followed by paralysis in the treated groups. Previous studies have shown that increased carbonyls have been detected in several cases such as skeletal muscle damage due to exhaustive exercise (Butterfield and Kanski, 2001; Touyz, 2004). Moreover, we detected a highly significant positive correlation (Correlation coefficient = 0.831, $P = 0.0001$) between PCC and NO in the treated insects. So, the increase in PCC might be in part due to the increase in the NO concentration (Table 2). NO readily reacts with superoxide ($O_2^{\bullet-}$) to form peroxynitrite ($ONOO^-$), a potent oxidant and nitrating agent capable of attacking and modifying proteins, as well as depleting antioxidant defenses (McCord, 2000).

In agreement with the previous studies (Barros et al., 1998; Tambourgi et al., 1998; Vera et al., 2000; Vera and Carlos, 2002), we detected an increase in the level of NO in the treated insects, especially in El-Agramia group (Table 1). In the present investigation, the secretion of NO may play a prominent protective role against the neurotoxic effects of MTX and MCa toxins. On the other hand, NO may inhibit mitochondrial Ca^{2+} uptake and increases the probability for opening the mitochondrial ATP-sensitive K^+ channel by modulating affinity to ATP (Rakhit et al., 2001). However, these events suggest the protective effect mediated by NO during hypoxia induced by the scorpion venom, high concentrations of NO may cause oxidative stress in the treated insects. An increase in extramitochondrial NO concentration accumulated within the hypoxic period may cause inhibition of cytochrome oxidase (complex IV of the respiratory chain). This in turn increases the probability for one electron transfer to oxygen at the level of ubiquinone (Coenzyme Q) (Becker et al., 1999). Subsequently, formation of superoxide anion radicals by the respiratory chain becomes enhanced. NO and superoxide anion radicals are converted to the highly reactive $ONOO^-$ by a diffusion-limited reaction that further induces the formation of a variety of reaction products (Bauer, 2000). Alternatively, superoxide anion radicals are catalyzed by superoxide dismutase (SOD) to H_2O_2 (Schild et al., 2003). Both pathways of superoxide anion radical reactions, the formation of H_2O_2 and the formation of $ONOO^-$, lead to a decrease in the activity of both SOD and CAT in the current study (Table 1). In the treated insects, the activity of both SOD and CAT were decreased by the elimination of superoxide anion radical and H_2O_2 respectively. McCord (2000) reported that the most efficient way to eliminate undesirable toxic species is by means of catalysis. Families of antioxidant enzymes have evolved for this purpose, including superoxide dismutases for the elimination of the superoxide radical and catalases.

4.2. Intraspecific variation in the pharmacological properties of S. m. palmatus venom

Discriminant Functions Analysis (Fig. 3) showed that scorpion venom of the Egyptian *S. m. palmatus* collected from different biotopes exhibits an intraspecific diversity in neurotoxic and cytotoxic effects. Interestingly, the present toxicological results are consistent with our molecular data on the level of genes and expressed protein of this scorpion species (Abdel-Rahman et al., 2009). Using the RAPD technique and SDS-PAGE of scorpion venom, we have found that the Southern Sinai populations (Sahab, El-Agramia and Rahaba) grouped together and were some distance away from the WMCD population. We suspect that a combination of local environmental conditions, geographical separation and genetic separation may play a major role in the intraspecific variation of venom of *S. m. palmatus* (Abdel-Rahman et al., 2009). Taken together, we can conclude that: (1) the venom from the different populations reflects clear differences in its potency; (2) the venom inhibited the activity of AChE followed by spastic paralysis in the treated insects; (3) *S. m. palmatus* venom has the ability to induce oxidative stress in the treated insect groups manifested by the increase in the level of NO and PCC, decrease the activities of both Cu/Zn-SOD and CAT; (4) the results presented here open the field for additional research, especially for the unidentified components of the venom of *S. m. palmatus* inhabiting different biotopes in Egypt.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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