Determination of median effective dose (ED$_{50}$) of scorpion antivenom against scorpion envenomation using a newly developed formula

Saganuwan Alhaji Saganuwan

Abstract

Background: About 50 species of scorpions cause fatal scorpionism worldwide. Most of these are members of the Buthidae family, and include, among others, *Mesobuthus eupeus*, *Androctonus crassicauda*, *Leiurus abdullahbayrami*, *Leiurus quinquestriatus*, *Tityus pachyurus* and *Androctonus australis*. Because high doses of scorpion venom and antivenom can cause death and hypersensitive reactions, there is a need to develop a formula that can be used to calculate both lethal and effective doses for scorpion venom and antivenom, respectively, thereby obviating the need for laboratory experiments.

Methods: In view of this, a literature search was carried out with the aim of modifying the formula (LD$_{50} = \frac{ED_{50}}{3} \times W_a \times 10^{-4}$) for calculation of the median lethal dose (LD$_{50}$) of scorpion venom and the ED$_{50}$ of antivenom. The human equivalent dose (HED) formula was assessed for extrapolation of LD$_{50}$ and ED$_{50}$ from animals to human for comparison and relevance with the new formula.

Results: The findings showed that the newly developed formula (LD$_{50} = ED_{50}^{1/3} \times W_a \times 10^{-4}$) yielded results that are very close to the reported values. Therefore, the newly developed and HED formulas can be used for calculation of LD$_{50}$ and ED$_{50}$ values for scorpion venom and antivenom, respectively.

Conclusion: The new formula yielded better results than the HED formula, confirming its predictive validity, precision, and reliability, thereby obviating the need for rigorous experiments and justifying the principles of reduction, refinement, and replacement (3Rs).

Keywords: antivenom, ED$_{50}$, human equivalent dose, LD$_{50}$, Scorpion, venom

1 | INTRODUCTION

Scorpions are members of the order Scorpiones, class Arachnida, subgroup Arthropoda. Their poisonous stings (termed scorpionism) have caused innumerable deaths, and scorpionism has raised serious concern worldwide because of its high incidence, prevalence, morbidity, and mortality.$^1$ About 2.3 billion people from Africa, the near and Middle-East and South India are at risk, and annual stings of over 1.2 million are reported, resulting in 3250 deaths, with higher severity and mortality among children.$^2$ However, mortalities have

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2018 The Authors. *Animal Models and Experimental Medicine* published by John Wiley & Sons Australia, Ltd on behalf of The Chinese Association for Laboratory Animal Sciences

wileyonlinelibrary.com/journal/ame2

Animal Model Exp Med. 2018;1:228–234.
decreased in countries that use antivenoms.3 Scorpionism caused by Tityus pachyurus polock is characterized by sialorrhea, respiratory distress, profuse sweating, ataxia, restlessness, somnolence, and hypoglycemia in mice. However, the antivenoms Bioclon and Butantan, produced in Mexico and Brazil, respectively, are very effective against T. pachyurus Pocone.4 The toxicity of the venom is related to the maturity and weight of the scorpion.5

Out of 1500 species of scorpions, 46-50 species are lethal. The number of dangerous species in the Buthidae family is significantly higher than in other families. Despite the fact that scorpion envenomations are a considerable health problem in tropical and subtropical regions of the world, treatment requires the use of specific antiserum. In 1909, Charles Todd produced a serum that was effective against Buthus quinquestriatus venom by immunizing horses using crude venom as an antigen.6,7 The treatment requires a high amount of antivenom to yield satisfactory neutralization,8 which can lead to adverse reactions. Hence there is a need to assess the median effective dose (ED50) of scorpion antivenom against the LD50 of the venom, with a view to eliminating or reducing post-treatment adverse reactions and obviating the need for laboratory experiments.

2 | METHODS

A random search of the literature, including journals, textbooks, books of abstracts, conference proceedings, and other periodicals, was conducted to establish the species of poisonous scorpions, their venoms, antivenoms, median lethal doses (LD50), median effective doses (ED50), their immunogenic reactions and the formulas used for calculation of both LD50 and ED50 values.6,7,9-14 The following four formulas were extracted.

\[
\text{Median Lethal Dose (LD50)} = \frac{ED50}{3} \quad (1)
\]

LD50 = ED50/3 \times Wa/Wa (Weight of animal incooperated) \quad (2)

LD50 = ED50 \times Wa \times 10^{-4} (10^{-4} = \text{Safety factor incooperated}) \quad (3)

LD50 = ED50^{1/3} \times Wa \times 10^{-4} (1/3 = \text{Therapeutic exponent}) \quad (4)

Based on the above formulas, the initial formula (LD50 = ED50 \times Wm \times 10^{-4}) developed by Saganuwan15 is therefore modified to (LD50 = ED50^{1/3} \times Wm \times 10^{-4}).

A one-third exponent was used to provide therapeutic scaling of dose translation from animal to animal.16 The new formula was used to estimate ED50 and LD50 values for venom from Mesobuthus eupeus, Androctonus crassicauda, Leirus abdullahbayrami, Hottentotta saulcyi, Leirus quinquestriatus and T. pachyurus and Androctonus australis. ED50 and LD50 values were also calculated using the human equivalent dose (HED) formulas, and the two sets of values were compared with the values reported in the literature. Animal-human and human-animal ED50 and LD50 values of T. pachyurus venom and antivenom were respectively calculated using the new and human HED formulas. HED is equal to the animal dose multiplied by the animal correction factor (Kw) divided by the human Km factor. The Km factor is body weight (kg) divided by body surface area (m²). Body weights and body surface areas of animals and humans were taken from Reagan-Shaw et al16 and USEPA.17 The ED50S for antivenom against M. eupeus, A. crassicauda and T. pachyurus have been established,6,8,9-11 but were recalculated using the new and HED formulas. The routes of administration are indicated in Table 1.

3 | RESULTS

Species of scorpions, experimental LD50s of scorpion venoms, therapeutic ED50 values of scorpion antivenoms and their calculated ED50 values are presented in Table 1. Androctonus australis is the most poisonous among the seven species, followed by T. pachyurus, A. crassicauda, L. quinquestriatus, M. eupeus, Leirus abdullahbayrami and H. saulcyi, in that order (Table 1). Species of animals, their bodyweights, BSAs, km factors, human equivalent doses, calculated LD50s for Tityus pachyurus venom and ED50 values of the antivenom are presented in Table 2.

4 | DISCUSSION

The new formula yielded higher estimated doses of scorpion antivenom. The severity of toxicity signs are directly related to LD50. The lower the LD50 value, the more severe the toxicity signs. Therefore, A. australis venom with an LD50 (0.5 ng/kg) is the most dangerous among all the species of scorpions followed by T. pachyurus (4.8 μg/kg) and A. crassicauda (15.45 μg/kg) venom. Our findings are corroborated by a report that Tityus stigmurus envenomation caused death in humans characterized by cardiogenic shock, pulmonary edema, and severe neurological symptoms.20 The effect of scorpion venom on the frequency, but not the amplitude, of spontaneous glycinergic synaptic potentials of inhibitory and excitatory presynaptic nerves.21 Scorpion venom toxicity increases in a dose-dependent fashion. The decreasing order of acute toxicity of A. australis, T. pachyurus, A. crassicauda, L. quinquestriatus, M. eupeus, L. abdullahbayrami and H. saulcyi venom shown in Table 1 agrees with a report indicating that members of Buthidae family are of medical importance.12

Androctonus crassicauda scorpion venom has active constituents that could induce a sustained activation of human monocytes, expressed as IL-12.22 The venom has a distinct molecular mass component, from which two peptides (Acra 1 and Acra 2) have been fully amino acid sequenced. The peptides are similar to known sodium channel-specific toxins of other scorpions.23 Scorpion antivenom has preventive, neutralizing, and curative properties against M. eupeus scorpionism if applied at optimum time, dose and route.9 The LD50 of A. crassicauda venom has been estimated as 1.1 mg/kg by electrical stimulation and 39.19 mg/kg by maceration of telson.10 This venom has the lowest elimination rate among all known scorpion venoms, making it highly toxic. The long half-life of the venom...
| S/no | Species of scorpion | Species of experimental animal | Average weight (g) | Reported LD$_{50}$ of scorpion venom | Reported ED$_{50}$ of antivenom | ED$_{50}$ by the new formula | ED$_{50}$ by HED formula | Reference(s) | Comments |
|------|---------------------|-------------------------------|-------------------|-------------------------------------|--------------------------------|--------------------------|-----------------------------|----------------|----------|
| 1    | *Mesobuthus eupeus* | Swiss abino mice Rabbit       | 20 ± 20           | 0.18 mg/kg i.m. 4.5 mg/kg           | 1 ml of *Androctonus crassicauda* neutralized 464 LD$_{50}$ of *M. eupeus* in mice | 4.5 mg/kg                 | 0.35 mg/kg 1.0 mg/kg       | 6,9           | ED$_{50}$ is unknown |
| 2    | *Androctonus australis* | Mice                          | 20.0 ± 1.0        | 0.5 mg/kg, 12 mg/kg, 0.25 mg/kg    | 16-18 µg                     | 6.3 ng-5 mg/kg            | 0.48 ng-0.39 mg/kg         | 18            | ED$_{50}$ is unknown |
| 3    | *Androctonus crassicauda* | Swiss albino mice             | 25.0 ± 1.0        | 0.27 mg/kg15.45 µg/kg i.c.v.1.1 mg/kg, 39.19 mg/kg i.v.35 µg/kg, sc.0.8-1.3 mg/kg | 1 ml of *A. crassicauda* antivenom neutralized 940 LD$_{50}$ of *A. crassicauda* venom in mice | 5.1 mg/kg14.6 µg/kg27.0 mg/kg i.v.26.0 µg/kg sc.7.4-11.5 mg/kg | 0.49 mg/kg0.44 µg/kg0.79 mg/kg2.61 mg/kg2.51 µg/kg0.74-1.11 mg/kg | 6,7,10,11 | ED$_{50}$ is unknown |
| 4    | *Leiurus abdullahbayrami* | Mice                          | 20.0 ± 20         | 0.19 mg/kg sc. —                   | —                            | 4.6 mg/kg                 | 0.36 mg/kg               | 11            | ED$_{50}$ is unknown |
| 5    | *Hottentotta saulcyi* | Mice                          | 20.0 ± 1.0        | 0.73 mg/kg sc. —                   | —                            | 7.1 mg/kg                 | 0.55 mg/kg               | 13            | ED$_{50}$ is unknown |
| 6    | *Leiurus quinquestriatus* | Rabbit                        | 2000 ± 200       | 0.16-0.5 mg/kg —                   | —                            | 0.93-1.4 mg/kg            | 0.33-0.50 mg/kg         | 19            | ED$_{50}$ is unknown |
| 7    | *Tityus pachyurus* | Swiss Webstar mice            | 19.0 ± 1.0        | 4.8 µg/kg                          | 330 µg/mL (Bioclon)292 µg/mL (Butantan) | 13.6 mg/kg                 | 1.0 mg/kg                | 4             | High chance of hypersensitivity reaction |

---: No available information.

*Highly toxic.*
serves the need for long-acting antivenom for venom neutralization. Envenomation by *L. abdullahbayrami* causes hyperexcitability, agitation, aggressive behavior, squeezing, fighting, tachypnea, weakness, convulsion, and death due to cardiac and respiratory failure in mice. Envenomation by *L. quinquestriatus* produced degranulation of eosinophils, fever, oedema of cerebrum and myocarditis in rabbits,14 The reports confirm the medical importance of members of the Buthidae family.

Bio-distribution of two purified toxic fractions of *M. eupeus* toxin in mice show rapid clearance of the compounds from blood and tissue, except for the kidneys, signifying that *M. eupeus* toxicity may not last long in the body. Dissociation of the toxin-channel complex during depolarization is determined by the difference between the electrical energies of the activated states of normal and toxin-modified channels. Injection of partially purified toxic fractions into rabbits gave rise to more potent antivenoms than those presently available, generated using whole venom, signifying that purification could reduce the dose of antivenoms needed. The antivenom for *A. crassicauda* venom, with an LD$_{50}$ (15.45 μg/kg) in mice, neutralized *Mesobuthus gibbosus* venom (LD$_{50}$ 20 μg/kg) in the Aegean region of Turkey.3 Thus, highly potent antivenom could be produced from about 238 telsons in 51 days.28

The calculated ED$_{50}$/LD$_{50}$ ratios for Butantan (292 μg/mL) and Bioclon (330 μg/mL) antivenoms and *titus* toxin, the toxic principle of *Tityus* species, are 222 and 272, respectively, which give equivalent weights of 21.5 and 24.3 kg, showing that Butantan (292 μg/mL) and Bioclon (330 μg/mL) antivenoms could be used effectively in the treatment of humans weighing 21.5–24.3 kg, and signifying that age must be considered in the treatment of *titus* toxin.27 However, the difference in severity of symptoms observed in children and adults may be due to differences in the pharmacokinetics of the toxin. *M. eupeus* venom can be neutralized by monovalent, polyvalent and anti-idiotype antivenoms, which are non-toxicants and can be used as a vaccine in people at risk of scorpion stings.30 Lack of a reported effective dose of antivenoms for *L. abdullahbayrami*, *L. quinquestriatus*, *H. saulcyi*, *M. eupeus* and *A. crassicauda* shows the need for specific antivenoms for a number of scorpion species.

Scorpion antivenoms are specific antigens, detoxified venoms, toxins, purified venom fractions, natural toxoids, recombinant toxins, synthetic peptides, and monoclonal and recombinant antibodies.7 Using peptides derived from the sequence of scorpion toxins, the penetration of antipeptide antibodies can neutralize the cognate venom.31 Turkish antivenom against *A. crassicauda* is effective against other species of scorpions. Minimum lethal dose and minimum effective dose were used to evaluate the effect of Turkish antivenom on *M. gibbosus* envenomation,18 suggesting the predictive validity, precision, and reliability of the new formula in envenomotherapy. Scorpion stings result in adult morbidity and pediatric mortality1 and the most lethal species are *Tityus serrulatus* and *Tityus bahiensis* in Brazil, *Centruroides sulfusus*, *Centruroides lioniapus*, and *Centruroides sculpturatus* in Mexico, *L. quinquestriatus*, *A. crassicauda*, *A. mauretanica*, *A. australis*, *A. amoreuni*, and *Buthus occitanus* in the Middle East and North Africa, *Parabuthus grauntatus* and *Parabuthus transvaalics* in South Africa, and *Mesobuthus tamulus* and *Palamneus swammerdams* in India.32–35

*Androctonus australis* has complex venom that contains cytotoxic principles with very rapid resultant fatal effects.36 Effective monoclonal antibodies (mAbs) specific to the α-neurotoxin 1 (Aah1) from *A. australis* hector venom have been reported,37 which also has recombinant toxin II with immunological and biological properties.

| S/no | Species      | Body weight (kg) | BSA (m$^2$) | $K_m$ factor | HED calculated LD$_{50}$ (μg/kg) | HED calculated ED$_{50}$ (μg/kg) | ED$_{50}$ by the new formula (μg/kg) |
|------|--------------|------------------|-------------|-------------|----------------------------------|----------------------------------|----------------------------------|
| 1    | Mouse        | 0.02             | 0.007       | 2.9         | 4.8                              | 13.6                             | 133.3                            |
| 2    | Hamster      | 0.08             | 0.02        | 4.0         | 3.48                             | 7.6                              | 75.7                             |
| 3    | Rat          | 0.15             | 0.025       | 6.0         | 2.32                             | 5.4                              | 53.7                             |
| 4    | Guinea pig   | 0.4              | 0.064       | 5.8         | 2.40                             | 3.9                              | 39.1                             |
| 5    | Rabbit       | 1.8              | 0.15        | 12.0        | 1.16                             | 1.9                              | 18.6                             |
| 6    | Monkey       | 3.0              | 0.24        | 12.5        | 1.11                             | 1.5                              | 15.5                             |
| 7    | Cat          | 7.0              | 0.37        | 18.9        | 0.74                             | 1.0                              | 10.0                             |
| 8    | Dog          | 10.0             | 0.50        | 20.0        | 0.70                             | 0.9                              | 8.9                              |
| 9    | Baboon       | 12.0             | 0.60        | 20.0        | 0.70                             | 0.8                              | 8.4                              |
| 10   | Ferret       | 0.30             | 0.043       | 7.0         | 1.99                             | 4.0                              | 40.5                             |
| 11   | Marmoset     | 0.35             | 0.06        | 5.8         | 2.40                             | 4.1                              | 40.9                             |
| 12   | Squirrel monkey | 0.6            | 0.09        | 6.7         | 2.08                             | 3.3                              | 32.6                             |
| 13   | Micro-pig    | 20.0             | 0.74        | 27.0        | 0.52                             | 0.6                              | 29.6                             |
| 14   | Mini-pig     | 40.0             | 1.14        | 35.1        | 0.40                             | 0.5                              | 21.5                             |
| 15   | Child        | 20.0             | 0.8         | 25.0        | 0.56                             | 0.7                              | 30.4                             |
| 16   | Adult human  | 60.0             | 1.6         | 37.5        | 0.37                             | 0.4                              | 4.0                              |

BSA: body surface area; HED: human equivalent dose formula; $K_m$: metabolism constant (body weight [kg] divided by body surface area).
In addition, A. australis hector venenation is mediated by cytokines and the complement system, which activate in turn to damage tissue. Kinins are also involved in cardiovascular toxicity and cause lethality of L. quinquestriatus venom in rabbits. A. australis garzonii venom (100 μg/kg) was neutralized by 4 mg/kg of antivenom injected intravenously. Antivenoms against a number of scorpion venoms have been reported, but the potency of antivenom in relation to the potency of scorpion venom should be investigated and both LD50 and ED50 should be determined paradoxically and canonically. The LD50 of intravenous venom from Vipera berus berus (0.4 μg/kg; symptoms included head-drop, floppy neck, flaccid paralysis of limb, respiratory paralysis, and death), Laticauda colubrine (0.05-0.13 μg/g), Sri Lankan Bungarus caeruleus (0.07 μg/g), Naja naja, B. caeruleus, Dabora russelli and Echis carinatus show that the scorpion venoms are highly toxic. Similar symptoms were observed for Vipera nikolskii venom (1.0 μg/kg), but the symptoms, caused by phospholipase A2, were lost after the mice were injected with strontium. Hence, strontium may be suitable as an antivenom against V. berus berus and A. australis venoms. The newly developed dot-ELISA for detection of the venoms of the Indian venomous snakes Naja naja, B. caeruleus, Dabora russelli and Echis carinatus and proteomic enzyme analysis may generally be used to detect scorpion venoms. The venoms of Montivipera raddei and Montivipera buljardahica, which have high levels of toxicity, have been shown to have potent cytotoxicity against AS49 human lung carcinoma, signifying that scorpion venom may also have anticancer properties. Lethal doses of L. quinquestriatus were 0.5 mg/kg i.v. and 3 μg/kg i.m.

The LD50 of T. pachyurus venom and the ED50 for its antivenom in monogastric animals are presented in Table 2. The results show that mouse (4.8 μg/kg) is the most sensitive to T. pachyurus venom followed by hamster (3.48 μg/kg), guinea pig (2.40 μg/kg), rat (2.32 μg/kg), rabbit (1.16 μg/kg), monkey (1.11 μg/kg), marmoset (2.40 μg/kg), squirrel monkey (2.08 μg/kg), ferret (1.99 μg/kg), cat (0.74 μg/kg), dog and baboon (0.70 μg/kg), child (0.56 μg/kg), micro-pig (0.52 μg/kg), mini-pig (0.40 μg/kg) and adult human (0.37 mg/kg) respectively, indicating that T. pachyurus venom is very toxic. The toxicity may be due to the presence of tityutoxins that are also present in T. pachyurus, T. stigmurus, Tityus abscurus and T. serralatus venom. The toxic principle acts via Na+, K+, Ca2+ and Cl− channels, signifying excitation of heart, CNS, and muscular fibres. The reported ED50s (0.4-13.6 μg/kg) for scorpion antivenoms for all the surveyed species using the HED formula are low, which may lead to therapeutic failure. The higher ED50s (4.0-133.3 μg/kg) for all the species calculated using the new formula indicate an improved neutralization potential. Based on these calculations, the mini-pig could be the best model for determination of LD50 and ED50 for T. pachyurus venom and antivenom, respectively.

5 CONCLUSION

This study showed that A. australis venom is the most toxic, followed by T. pachyurus, A. crassicauda, L. quinquestriatus, M. euepus, L. abdullabayrami, and H. sauleyi. The modified and HED formulas can be used to estimate the LD50 and ED50 values of the scorpion venoms and antivenoms, respectively. The newly developed formula, incorporating safety factor, animal weight and therapeutic index, yielded increased quantities of scorpion antivenoms, that should adequately neutralize the scorpion venoms, obviating the need for laboratory experiments and reducing the risk of hypersensitivity to the antivenoms.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

SA Saganuwan conceived the idea and wrote the manuscript.

ORCID

Saganuwan Alhaji Saganuwan http://orcid.org/0000-0002-0963-5569

REFERENCES

1. Santos MS, Silva CG, Neto BS, et al. Clinical and epidemiological aspect of scorpionism in the world: a systematic review. Wilderness Environ Med. 2016;27(4):504-518.
2. Chippaux JP, Goyffon M. Epidemiology of scorpionism: a global appraisal. Acta Trop. 2008;107(2):71-79.
3. Chippaux JP. Epidemiological investigation on envenomation: from theory to practice. J Venom Anim Toxins Incl Trop Dis. 2012;18(4):446-450.
4. Barona J, Otero R, Núñez V. Toxicological and immunological aspects of scorpion venom (Tityus pararchyurus) neutralizing capacity of antivenom produced in Latin America. Biodoncia. 2004;24(1):42-49.
5. Ozkan O, Adiguzel S, Kar S, Yakistiran S, Cesaretli KY, Karaer KZ. Determination of potency and paraspecific effects of Androctonus crassicauda (Oliver, 1807) antivenom against Mesobuthus gibbosus (Brulle, 1832) venom (Scorpiones: Buthidae). J Venom Anim Incl Trop Dis. 2007;13(2):500-508.
6. Ozkan O, Carhan A. The neutralizing capacity of Androctonus crassicauda antivenom against Mesobuthus euepess scorpion venom. Toxicon. 2008;52(2):375-379.
7. Carro AO, Chatzaki M, Horta CC, et al. Alternative methodologies of scorpion antivenom production. Toxicon. 2015;97:64-74.
8. Ismail M. The scorpion envenoming syndrome. Toxicon. 1995;33(7):825-858.
9. Zayerzadeh E, Koohi MK, Mirakabadi AZ, et al. Amelioration of cardio-respiratory perturbations following Mesobuthus euepes envenomation in anesthetized rabbits with commercial polyvalent F(ab’2) antivenom. Toxicon. 2012;59(2):249-256.
10. Ozkan O, Kar S, Güven E, Ergun G. Comparison of proteins, lethality and immunogenic compounds of Androctonus crassicauda (Oliver, 1807) (Scorpiones: Buthidae) venom obtained by different methods. J Venom Anim Incl Trop Dis. 2007;13(4):844-856.
11. van Zoelen SA, Ozkan O, Inceoglu B. Antegenic cross-reactivity antibotoxin antibody against Androctonus crassicauda venom. J Arthropod Borne Dis. 2015;9(2):176-183.
12. Ozkan O, Yagmur EA, Ark M. A newly described scorpion species Leirus abdullahiabayarani (Scorpion: Buthidae), and the lethal potency and in vivo effects of its venom. J Venom Anim Toxins Incl Trop Dis. 2011;17(4):414-421.

13. Yagmur EA, Ozkan Ö, Kavaer KZ. Determination of the median lethal dose and electrophoretic pattern of Hottentotta saulaji (Scorpiones: Buthidae) scorpion venom. J Arthropod Borne Dis. 2015;9(2):238-245.

14. Afifi SH, Elkashef AS, Salem DA. Light and transmission electron microscopical changes associated with Leirus quinqueniatrus venom in rabbits. Macedonian Vet Rev. 2010;39(1):51-57.

15. Saganuwan SA. The new algorithm for calculation of median lethal dose (LD50) and effective dose fifty (ED50) of Micurus fulvius venom and anti-venom in mice. Int J Vet Sci Med. 2016;4(1):1-4.

16. Reegan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J. 2008;22(3):659-661.

17. US Environmental Protection Agency. (USEPA). Guideline for cardiovascular after scorpion envenomation. 2011;17(4):414.

18. Gueron M, Ilia R, Sofer S. The cardiovascular after scorpion envenomation. A review. J Toxicol Clin Toxicol. 1992;30(2):245-258.

19. Mozhaeva GN, Naumov AP. Knietics of interaction of scorpion with Androctonus crassicauda venom. J Venom Anim Toxins Incl Trop Dis. 2006;12(3):390-399.

20. Delari P, van Rietschoten J, Rochat H. Scorpion venoms and neurotoxicity. A review. Neurophysiology. 1989;40(1):89-117.

21. Alzeid TA, Al-Saadi M, Assarehzadegan MA, Pipelzadeh MH, Hadaddezfuli R. Comparison of two purified toxic fractions from Androctonus australis venom. Toxicon. 2011;57:426-436.

22. Caliskan F, García BI, Coronas FI, Batista CV, Zamudio FZ, Possani LD. Characterization of venom components from the scorpion Androctonus crassicauda of Turkey. Toxicon. 2006;48(12):122.

23. Ismail M, Abdelsalam M, Al-Ahaidib MS. Androctonus crassicauda (Oliver), a dangerous and unduly neglected scorpion – I Pharmacological and clinical studies. Toxicon. 1994;32(12):1599-1616.

24. Shimrandi SP, Shamsaei M, Gandomkar M, Sarie E, Ghannadi M, Zare A. Comparison of two purified toxic fractions from Mesobuthus eupeus scorpion venom. J Venom Anim Toxins Incl Trop Dis. 2010;16(4):639-646.

25. Mozhavea GN, Naumov AP. Kinetics of interaction of scorpion venom with sodium channels on the Ranvier node membrane. Neurophysiology. 2010;52(2):619-626.

26. Delari P, van Rietschoten J, Rochat H. Scorpion venoms and neurotoxins: an immunological study. Toxicon. 1981;19(3):393-407.

27. Numan EA, Arya V, Hochhaus G, Cardsos VN, Moraes-Santos T. Age effects on the pharmacokinetics of Tityus serrulatus venom in rats. Braz J Med Biol Res. 2004;37(3):385-390.

28. Khodadel M, Zahraei-Salehi T, Nayeri-Fasaei, B, et al. Purification of the immunogenic fractions and determination of toxicity in Mesobuthus eupeus (Scorpionida: Buthidae) Venom. J Arthropod Borne Dis. 2013;7(2):139-146.

29. Alvarenga LM, Dining CR, Grainer C, Chávez-Olórtegui C. Induction of neutralizing antibodies against Tityus serrulatus scorpion toxins by immunization with a mixture of defined synthetic epitopes. Toxicon. 2002;40(1):89-95.

30. Salama MW, Sharshar KM. Surveillance study on scorpion species in Egypt and comparison of their crude venom protein files. J Basic Appl Zool. 2013;66(2):76-86.

31. Balozet L. Scorpionism in the Old World. In: Bucherl W, Buckelry EE, eds. Venomous Animals and Their Venoms, 1st edn. New York: Academic Press; 1971:379-381.

32. Theakston RD, Warrell DA, Griffiths E. Report of a WHO workshop on the standardization and control of antivenoms. Toxicon. 2003;41(5):541-557.

33. Nafie MS, Daim MMA, Ali IAI, Abdel-Rahman MA, Nabil ZI. Pharmacological and biochemical characterization of the Egyptian scorpion “Androctonus australis” venom. Abstract of 6th International Conference on Natural Toxins, Ismailia, December, 2014:1-2.

34. Clot-Faybesse O, Juin M, Rochat H, Devaux C. Monoclonal antibodies against the Androctonus australis hector scorpion neurotoxin 1: characterization and use for venom neutralisation. FEBS Lett. 1999;458(3):313-318.

35. Bougis PE, Rochart H, Smith LA. Precursors of Androctonus australis scorpion neurotoxins. Structures of precursors, processing outcomes, and expression of a functional recombinant toxin II. J Biol Chem. 1989;264(32):19259-19265.

36. Adi-Bessalem S, Hammoudi-Triki D, Laraba-Djebari F. Pathophysiological effects of Androctonus australis hector scorpion venom: tissue damages and inflammatory response. Exp Toxicol Pathol. 2008;60(4-5):373-380.

37. Fatani AJ, Furman BL, Zeilin II. The involvement of plasma kinins in the cardiovascular effects of Leirus quinqueniatrus scorpion venom in anaesthetised rabbits. Toxicon. 1998;36(3):523-536.

38. Krifi MN, Savin S, Debray M, Bon C, El Ayeb M, Choumet V. Pharmacokinetic studies on scorpion venom before and after antivenom immunotherapy. Toxicon. 2005;45(2):187-198.

39. Laustsen AH, Solå M, Jappe EC, Oscoz S, Lauridsen LP, Engmark M. Biotechnological trends in spider and scorpion antivenom development. Toxins (Basel). 2016;8(8):1-13.

40. Saganuwan SA, Onyeyili PA. The paradox of human equivalent dose formula: a canonical case study of Abrus precatorius aqueous leaf extract in mangostric animals. Mac Vet Rev. 2016;39(1):23-32.

41. Malina T, Krecsák L, Westerström A, et al. Individual variability of venom from the European adder (Vipera berus berus) from one locality in Eastern Hungary. Toxicon. 2017;135:59-70.

42. Tan CH, Wong KY, Tan KY, Tan NH. Venom proteome of the yellow-lipped sea krait, Laticauda colubrina from Bali: insights into subvenom diversity venom antigenicity and cross-neutralization by antivenom. J Proteomics. 2017;166:48-58.

43. Amf O, Tan CH, Ariaianee GC, Quraishi N, Tan NH. Venomics of Bungarus caeruleus (Indian Krait): comparable venom profiles, variable immune re-activities among specimens from Sri Lanka, India and Pakistan. J Proteomics. 2017;164:1-18.

44. Tan NH, Wong KY, Tan CH. Venomies of Naja sputatrix, the Javan-spitting cobra: a short neurotoxin-driven venom needing improved antivenom neutralization. J Proteomics. 2017;157:18-32.

45. Shaikh IK, Dixit PP, Pawade BS, Waykar IG. Development of dot-ELISA for the detection of venoms of major Indian venomous snakes. Toxicon. 2017;139:66-73.

46. Choudhury M, McCleary RJR, Keshervani M, Kini RM, Velmurugan D. Comparison of proteomic profiles of two of the Big four snakes of India, the Indian cobra (Naja naja) and the common krait (Bungarus caeruleus) and analyses of their toxins. Toxicon. 2017;135:33-42.
with potent cytotoxicity against human A549 lung carcinoma cells. Toxicol. 2017;135:71-83.

51. Fatani AJ. Comparative study between peripherally and centrally acting sublethal and lethal doses of Leiurus quinquestriatus scorpion venom in rabbits: the usefulness of the sodium channel blocker lidocaine. Saudi Pharm J. 2000;18(3):137-151.

52. Batista CV, Román-González SA, Salas-Castillo SP, Zamudio FZ, Gómez-Lagunas F, Possani LD. Proteomic analysis of the venom from the scorpion Tityus stigmurus: biochemical and physiological comparison with other Tityus species. Comp Biochem Physiol C Toxicol Pharmacol. 2007;146(1-2):147-157.

53. Pimenta AM, Stöcklin R, Favreau P, Bougis PE, Martin-Eauclaire MF. Moving pieces in a proteomic puzzle: mass finger printing of toxic fractions from the venom of Tityus serrulatus (Scorpiones, Buthidae). Rapid Commun Mass Spectrom. 2001;15(17):1562-1572.

54. Guerrero-Varga JA, Mourao CBF, Quintero-Hernandez V, Possani LD, Schwartz EF. Identification and phylogenetic analysis of Tityus pachyurus and Tityus obscurus novel putative Na⁺ – channel scorpion toxins. PLoS ONE. 2012;7(2):e30478.

55. Barona J, Batista CV, Zamudio FZ, et al. Proteomic analysis of the venom and characterization of toxins specific for Na⁺ – and K⁺ – channels from the Colombian scorpion Tityus pachyurus. Biochim Biophys Acta. 2006;1764(1):76-84.

How to cite this article: Saganuwan SA. Determination of median effective dose (ED₅₀) of scorpion antivenom against scorpion envenomation using a newly developed formula. Animal Model Exp Med. 2018;1:228–234. https://doi.org/10.1002/ame2.12031