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ORIGINAL ARTICLE

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

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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

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* Pocock.[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 (ED_{50}) of scorpion antivenom against the LD_{50} 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 (LD_{50}), median effective doses (ED_{50}), their immunogenic reactions and the formulas used for calculation of both LD_{50} and ED_{50}.[6,7,9-14] The following four formulas were extracted.

$$\text{Median Lethal Dose}\,(LD_{50}) = ED_{50}/3 \quad (1)$$

$$LD50 = ED_{50}/3 \times W_a (W_a = \text{Weight of animal incooperated}) \quad (2)$$

$$LD50 = ED_{50} \times W_a \times 10^{-4} (10^{-4} = \text{Safety factor incooperated}) \quad (3)$$

$$LD50 = ED_{50}1/3 \times W_a \times 10^{-4} (1/3 = \text{Therapeutic exponent}) \quad (4)$$

Based on the above formulas, the initial formula ($LD_{50} = \frac{ED_{50}}{3} \times W_m \times 10^{-4}$) developed by Saganuwan[15] is therefore modified to ($LD_{50} = {ED_{50}}^{1/3} \times W_m \times 10^{-4}$).

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 ED_{50} and LD_{50} values for venom from *Mesobuthus eupeus*, *Androctonus crassicauda*, *Leiurus abdullahbayrami*, *Hottentotta saulcyi*, *Leiurus quinquestriatus* and *T. pachyurus* and *Androctonus australis*. ED_{50} and LD_{50} 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 ED_{50} and LD_{50} 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 (K_m) divided by the human K_m factor. The K_m factor is body weight (kg) divided by body surface area (m^2). Body weights and body surface areas of animals and humans were taken from Reagan-Shaw et al[16] and USEPA.[17] The ED_{50}s for antivenom against *M. eupeus*, *A. crassicauda* and *T. pachyurus* have been established,[4,6,9-11] but were recalculated using the new and HED formulas. The routes of administration are indicated in Table 1.

3 | RESULTS

Species of scorpions, experimental LD_{50}s of scorpion venoms, therapeutic ED_{50} values of scorpion antivenoms and their calculated ED_{50} 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*, *Leiurus abdullabayrami* and *H. saulcyi*, in that order (Table 1). Species of animals, their bodyweights, BSAs, km factors, human equivalent doses, calculated LD_{50}s for *Tityus pachyurus* venom and ED_{50} 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 LD_{50}. The lower the LD_{50} value, the more severe the toxicity signs. Therefore, *A. australis* venom with an LD_{50} (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 and glutamatergic postsynaptic currents suggests that scorpion toxin acts on 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* venoms 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 LD_{50} 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

TABLE 1 The species of scorpions, average weights of experimental animals, median lethal dose (LD_{50}) and effective dose 50 (ED_{50}) of scorpion venoms and antivenoms

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 ± 2.0 200 ± 2.0	0.18 mg/kg[a] i.m. 4.5 mg/kg	1 ml of *Androctonus crassicauda* neutralized 464 LD_{50} of *M. eupeus* in mice	4.5 mg/kg 2.82 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[a], 12 mg/kg[a], 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[a] i.c.v.1.1 mg/kg, 39.19 mg/kg i.v.35 μg/kg[a] 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/kg4.6 μg/kg8.2 mg/kg27.0 mg/kg i.v.26.0 μg/kg sc.7.4-11.5 mg/kg[2]	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 ± 2.0	0.19 mg/kg[a] 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[a]	—	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[a]	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.
[a]Highly toxic.

TABLE 2 Mouse-human and human-other animals extrapolated median lethal dose (LD_{50}) and effective dose 50 (ED_{50}) of *Tityus pachyurus* venom and antivenom

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).

suggests the need for long-acting antivenom for venom neutralization.[24] Envenomation by *L. abdullahbayrami* causes hyperexcitability, agitation, aggressive behavior, squeaking, fighting, tachypnea, weakness, convulsion, and death due to cardiac and respiratory failure in mice.[12] Envenomation by *L. quinquestriatus* produced degranulation of eosinophils, fever, oedema of cerebrum and myocarditis in rabbit.[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,[25] 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 electrical energies of the activated states of normal and toxin-modified channels.[26] Injection of partially purified toxic fractions into rabbits gave rise to more potent antivenoms than those presently available, generated using whole venom,[27] 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,[10] neutralized *Mesobuthus gibosus* venom (LD_{50} 20 μg/kg) in the Aegean region of Turkey.[6] 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 tityus toxin, the toxic principle of *Tityus* species, are $\frac{292}{13.6}$ and $\frac{330}{13.6}$, 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 tityus toxin.[29] 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 mortality[1] and the most lethal species are *Tityus serrulatus* and *Tityus bahiensis* in Brazil, *Centruroides suffusus*, *Centruroides lionpidus*, and *Centruroides sculpturatus* in Mexico, *L. quinquestriatus*, *A. crassicauda*, *A. mauretanicus*, *A. australis*, *A. amoreuni*, and *Buthus occitanus* in the Middle East and North Africa, *Parabuthus grauntatus and Parabuthus transvaalicus* in South Africa, and *Mesobuthus tamulus* and *Palamneus swammerdance* 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.[38]

In addition, *A. australis* hector envenomation is mediated by cytokines and the complement system, which activate in turn to damage tissue.[39] Kinins are also involved in cardiovascular toxicity and cause lethality of *L. quinquestriatus* venom in rabbits.[40] *A. australis garzonii* venom (100 μg/kg) was neutralized by 4 mg/kg of antivenom injected intravenously.[41] Antivenoms against a number of scorpion venoms have been reported,[42] but the potency of antivenom in relation to the potency of scorpion venom should be investigated[12] and both LD_{50} and ED_{50} should be determined paradoxically and canonically.[43] The LD_{50}s 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),[44] *Laticauda colubrine* (0.05-0.13 μg/g),[45] Sri Lankan *Bungarus caeruleus* (0.07 μg/g),[46] *Naja sputatrix*[47] and *A. australis* (0.5 ng)[18] 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 A_2, were lost after the mice were injected with strontium.[18] 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*[48] and proteomic enzyme analysis[49] may generally be used to detect scorpion venoms. The venoms of *Montivipera raddei* and *Montivipera bubjardahica*, which have high levels of toxicity, have been shown to have potent cytotoxicity against AS49 human lung carcinoma,[50] signifiying 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.[3]

The LD_{50} of *T. pachyurus* venom and the ED_{50} 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. serrulatus* venom. The toxic principle acts via Na^+, K^+, Ca^{2+} and Cl^- channels, signifying excitation of heart, CNS, and muscular fibres.[51-55] The reported ED_{50}s (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 ED_{50}s (4.0-133.3 μg/kg) for all the species calculated using the new formula indicate an improved neutralization potential.[4] Based on these calculations, the mini-pig could be the best model for determination of LD_{50} and ED_{50} 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. eupeus*, *L. abdullabayrami*, and *H. saulcyi*. The modified and HED formulas can be used to estimate the LD_{50} and ED_{50} 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.

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