Chapter

Toxicosis of Snake, Scorpion, Honeybee, Spider, and Wasp Venoms: Part 2

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Abstract

Toxicosis is a poisoning caused by venomous animals such as snake, scorpion, honeybee, spider and wasp. Their poisons contain amino acids, peptides, proteins, enzymes and metallic ions that are responsible for neurotoxicity, hemotoxicity and myotoxicity. Because of in vivo therapeutic challenges posed by toxicosis, there is need for ideal therapeutic agents against envenomation caused by venomous animals. Findings have shown that toxicosis could be treated symptomatically. Snake and scorpion antivenins could be used for treatment of poisoning caused by snake, scorpion, honeybee, spider and wasp. The amount of antivenin is dependent on the quantity of venom injected into the affected individuals. Moreso, sympotomatic treatments are also done according to the systems affected. Hospitalization is necessary for assessment of therapeutic success.

Keywords: toxicosis, snake, scorpion, toxin, antivenin, lethality, hemotoxicity, neurotoxicity, myotoxicity, hospitalization

1. Introduction

Venomous animals such as snake, scorpion, honeybee, spider and wasp constitute very significant health hazard in the world. The snake venom contains many toxic and non-toxic molecules [1]. Fourty-seven out of 50 US States have venomous snakes. Southwestern US are mostly affected. About 4700 venomous snakes bite human and 150,000 primarily dogs and cats are bitten by venomous snakes every year in the US, with human mortality of 0.06% and that of dog is 1–30% [2]. Scorpionism is caused by many poisonous scorpions including Tityus species endemic to Panama whereas latruroides are endemic to Guatemala, Belize, El Salvador, Nicaragua, and Costa Rica. They are wildly toxic via unchannel active toxins. In Panama the incidence was 52 cases per 100, 000 in 2007 and 28 deaths were recorded between 1998 and 2006 respectively. Tityus species present in the Atlantic coast of Costa Rica is responsible for fatalities in Panama. *Tityus pachyurus* [3] and *Parabutus granulatus* are of the most medical importance in the Western Cape of South Africa. *P. transvaalicus* venom is used for production of *P granulatus* venom [4]. About 200, 000 cases of scorpionism are reported in Mexico and cause 310 deaths every year and 20, 000 out of 38, 068 affected persons were successfully treated using equine antiserum (serotherapy) and no life was lost [5]. The first case of scorpionism was reported in Canada in a 36 year old man in 1962. The rate of scorpionism in Amazon region of Brazil is 8.14–273 cases

per 100,000. Most species involved in envenomation belong to the genus of Tityus [6]. *M. gibbosus* is endemic to a small geographic area of Erbalj on terra rossa soil [7]. The incidence of scorpion sting in Iran was 61.2 per 100,000 populations [8], as against 1.2 million people with estimated 3250 death per year. The mean annual rate was 17.4 per woman population [9]. The highest rate of sting occurred in Iran among individuals of 25–34-year-old [8]. The global mortality rate was 10 per 1000 cases. Most of the stings were seen on lower limbs (58.6%) and upper limbs (34.3%) during the hot season [10]. The scorpion that had envenomated for the first time may have less toxic envenomation for the second time as reported in the case of sting from *Leiurus abdullahbayrami* [11]. In India envenomation was more common in males than in females [12].

Honeybee (*Apis mellifera*) constitutes a significant nuisance and of medical importance in Africa, Europe and other parts of the world. Other subspecies are *A. mellifera carnica*, *A. mellifera ligustica* and *A. mellifera scutellata* [13]. Honeybee stings reported in Ceare, Brazil showed 1307 cases affecting men between 20 and 29 years of age [14] translating to 19 cases per 100,000 in Campina Grade [15]. Bee envenomation is a problem in India, China, Latin America, Middle East, North and South Africa [16]. About 200 stings from *Apis mellifera* could cause envenoming syndrome in children and elderly [17] as multiple stings, not increased venom potency or delivery cause serious reactions [18]. Bee venoms differ in weight, concentrations of phospholipase and melittin [19]. Unfortunately, no specific antivenom for bee envenomation, hence proper removal of stings, first aid treatment and chemotherapy should be considered as medical emergency [20]. Administration of hydrocortisone, calcium, analgesic, 0.9% sodium chloride and application of ice to the site of stung pregnant woman resulted in recovery from the pain and the fetus was stable and delivered 3 months after treatment without sequela [21]. There are 42,473 species of spiders grouped into 110 families (Platnick,) [22]. *Hadronyche formidabilis* and *H. cerberea* have very high envenoming rates [23]. Black widow spider (*Latrodectus mactans*), brown recluse spider (*Loxosceles reclusa*) are of most concern [24]. In view of the increased challenges and negligence of envenomotion caused by venomous snake, scorpion, spider, wasp and bee, there is need for thorough search for their therapeutic regimens with a view to having lasting solution against fatality.

2. Methodology

Literatures were searched on venomous snakes, scorpions, honeybees, spiders and wasps with an intent to identifying their toxicity potentials, epidemiology of their toxicosis, signs of toxicity, treatment and development of vaccines against their venoms. Sought also are information on medicinal plants, phytochemicals and other therapeutic agents, structures of some chemicals present in the venoms, their medical applications and medicinal uses. Mathematical formulas were also derived for calculation of body weight, body surface area, packed cell volume, hemoglobin, total blood volume, lost blood volume, median lethal dose (LD_{50}),median effective dose (ED_{50}), number of bee stings, total dose of bee venom and relationship between renotoxicity and haemotoxicity.

3. Results

Findings have shown that venoms from poisonous species of snakes, scorpions, honeybees, spiders and wasps are highly toxic and could cause various degrees of hemotoxicity, myotoxicity, and neurotoxicity including death (**Tables 1** and **2**).

Animal	Weight (kg)	Number of stings	Dose of stings (mg)	LD_{50} (mg/kg)	ED_{50} (mg/kg)	Lethal time (hr)
Child	10	94	28.2	1.41	11.2	2.10
Adult	60	1000	300	2.5	7.5	3.72

Table 1.
Calculated median lethal dose and effective dose fifty of honeybee venom and antivenom in human.

System	Age (yr)	Sting(s)	Sex	Signs	Treatment
Nervous	6,70 70	Each 1	Female Male	Axonal polyneuropathy, seizure brachial plexitis, Parkinsonism	Immunoglobulin I.V., anti-inflammatory, Oxygen
Muscular	34	3	Male	Rhadbomyolysis, muscle pain, allergy	Analgesic, anti-inflammatory
Circulatory	35–42	Many	Male	Myocardial infarction, ischemic attack, cardiac arrest, anaphylaxis, kounis syndrome	Angioplasty, steroids, antihistaminics
Renal	2	1	Male	Nephrotic syndrome, anasarca, acute renal failure	Corticosteriod
Ocular	21–50	1	Male Female	Cataract, glaucoma	Surgery, keratoplasty, antibiotics, corticosteroid
Miscellaneous	40	Multiple	Male	Intravascular coagulopathy, coma	Hydrocortisone, analgesic, pheniramine

Table 2.
Toxicity signs of honeybee sting in human.

4. Discussion

4.1 Treatment of scorpion envenomation requires body weight

Recently Abimannane *et al reported* that treatment of children showing autonomic symptoms from Indian red scorpion envenomation that could lead to myocardial infarction required second dose of 30 mg (3 ml) [25]. However, reference was not made to the weight of the children. Hence, the scorpion antiserum against Indian red scorpion (*Mesobuthus tamulus*) was reassessed using a child that weighed 20 kg body weight. Findings have shown that 12 mg (1.2 ml) should be given to the affected child (20 kg). Therefore, administration of the scorpion antivenin should be dose dependent to avoid death and hypersensitivity reaction that could be caused by *M. tamulus* venom and antivenin. The newly derived formula by Saganuwan is very relevant in the present context. Venom-antivenin neutralization factor of 1:2 to 1:25 could be tried. Fatal scorpionism is caused by members of Buthidae and Mesobuthus species is highly poisonous in human. The LD_{50} and ED_{50} of venom and antivenin of Mesobuthus species in mice are 0.18 mg/kg and 2.82 mg/kg respectively. High doses of venom and antivenin cause death and hypersensitivity reactions [26]. Therefore, there is need to reassess treatment of *Mesobuthus tamulus* envenomation in children. The formula developed by Saganuwan [26] was used to determine the LD50 and ED_1 of *Mesobuthus tamulus* venin and antivenin in a 20 kg weighed child.

$LD_{50} = ED_{50}^{1/3} X\ W_h x 10^{-4.}$

$LD_{50} = ED_{50}^{1/3}$ X Wh x 10^{-4} (Wh = Weight of human).

$LD_{50} = 30^{1/3}$ x 20,000x 10^{-4} (Ikg = 1000 g).

$LD_{50} = 30^{1/3}$ x 20x 10^{4}x 10^{-4}.

= $30^{1/3}$ x 2.
= 3.11 x 2 = 6.22 mg/20 kg.
30 mg → 50 children.
X → 1child

$$x = \frac{30x1}{50} = \frac{3}{5} = 0.6\, mg/kg$$

:. 20 x 0.6 = 12 mg should be given instead of 30 mg.

The calculated LD_{50} (6.22 mg) per 20 kg weighed child translating to 0.31 mg/kg shows that *M. tamulus* venom is very toxic. Moreso the calculated effective dose (0.6 mg/kg) of *M. tamulus* antivenin shows that neutralization dose of the antivenin should be twice the dose of venom. The obtained 12 mg (1.2 ml) of the scorpion antivenin disagrees with the report of Abimannane *et al* [25] indicating that the initial dose of the antivenin should be 30 mg (3 ml) followed by 60 mg (6 ml) of the second dose. The second dose should be 24 mg (2.4 ml). Amelioration of cardio-respiratory perturbations caused by *M. eupeus* envenomation in mice using polyvalent F $(ab^1)_2$ antivenin proves that neutralization factor is 1:2 to 1:25 in mice [26]. Cardio-respiratory activity modifying agents including prazosin could be administered [27]. *Buthus quinqestriatus* venom was neutralized by an effective serum obtained from horses immunized with crude venom [28, 29]. High amount of antivenin is required for satisfactory neutralization. *Mesobuthus gibbosus* venom could be neutralized by *Androctonus crassicauda* antivenin [30]. Scorpionism affects severely children of up to 24.3 kg body weight [31]. *Mesobuthus* venom could be neutralized by non-toxicant antivenins which could be used as vaccines against scorpion envenomation [32]. The efficacy of the treatment depends on species of scorpion, dose, potency and route of administration of antivenin [33].

4.2 The role of exponent and lethality time in determination of death caused by toxins in animal models of experiment

Drugs, chemicals, plant extracts and venoms from snake, scorpion and honeybee could cause death when introduced in large quantities and the toxicity signs affect all the organ systems and, in many occasions, culminating in death. The use of large number of animals for determination of median lethal dose (LD_{50}) has been discouraged worldwide. Therefore based on the principle of R3 (Reduction, Refinement and Replacement), the number of animals for LD_{50} determination has been reduced to 2–6 and yielded good results in monogastric animals. The traditional method that involves the use of 40 animals was applied in the production of vaccine made from silicate against *Crotalus atrox*, *Agkistrodon contortrix contortrix* and *Agkistrodon piscivorus leucostoma*. The LD_{50} software was generated on the NNTRC home page (ntrc.tamuk.edu/LD50calculator.xls) based on the Saganuwan's method [34] unfortunately it is not accessible. Hence I am appealing to NNTRC to make it available for scientific use. Newly devised LD_{50} formulas were used to determine LD_{50} and ED_{50} for snake, scorpion and honeybee venoms and antivenoms respectively using 6 mice each. The modified LD_{50} formulas used comprised effective dose fifty (ED_{50}) divided by the denominator (3) for snake and raised to exponent (0.33) for scorpion and honeybee showing that the relationship between snake venom and scorpion or honeybee venom is the difference between the denominator (3) and exponent (0.33). Hence snake venom is more toxic than the scorpion and honeybee venoms. The toxicity effect of scorpion is much higher than that of honeybee whose toxicity depends on the number of stings; hence death may be delayed giving rise to lethality time that has exponent (0.33). Median lethal time (LT_{50}) is LD_{50} over D^P whereas D (dose) and P (exponent) are integral parts of the formula [26, 34, 35].

4.3 Hematological and renal parameters of animals envenomated by snakes

Hemotoxic and renotoxic effects of ophidiotoxicity have made quick treatment of snake envenomation difficult [36]. However, Saganuwan and Onyeyili reported that total volume of animal blood was 8% of its weight [36]. Saganuwan also reported relationship between ED_{50}, LD_{50} and body weight as a function of effective snake antivenom therapy as proven by neutralization of American pit viper envenomation [35]. Relationship between age, body weight, serum creatinine, plasma creatinine, ED_{50}, LD_{50} and safety factor has been established [37]. Hence, the formulas have been integrated for calculation of hemotoxic and renotoxic parameters of snake envenomation.

4.4 Relationship between renotoxicity and hemotoxicity of toxins

The formula established for calculation of ED_{50} and LD_{50} with safety factor in animals is:

$$\mathrm{LD}_{50} = \frac{ED_{50}}{3}\, x\, W_a\, x\, 10^{-4} \tag{1}$$

$$0.08(W_a) = \mathrm{TBV} \tag{2}$$

$$\mathrm{W_a} = \frac{TBV}{0.08} \tag{3}$$

Substitute for W_a in equation (1)

$$\therefore \mathrm{LD}_{50} = \frac{ED_{50}}{3}\, x\, \frac{TBV}{0.08}\, x\, 10^{-4} \tag{4}$$

$$\text{But TBV} = \text{PV} + \text{EV} \tag{5}$$

Substitute for TBV in equation (4)

$$\therefore \mathrm{LD}_{50} = \frac{ED_{50}}{3}\, x\, \frac{PV + EV}{0.08}\, x\, 10^{-4} \tag{6}$$

$$\mathrm{LD}_{50} = \frac{ED_{50}}{3}\, x\, (PV + EV)\, x\, 8\, x\, 10^{-2} \tag{7}$$

$$\text{But Hb} = 0.33\ (\text{EV}) \tag{8}$$

$$\therefore \text{EV} = \frac{Hb}{0.33} \tag{9}$$

Substitute for EV in equation (7)

$$\therefore \mathrm{LD}_{50} = \frac{ED_{50}}{3}\, x\, \left(PV + \frac{Hb}{0.33}\right) x\, 8\, x\, 10^{-2} \tag{10}$$

$$\text{However, CrCl} = \frac{K\, x\, (140 - age) x\, W_a}{D\, x\, Scr\, x\, 72} \tag{11}$$

$$\mathrm{W_a} = \frac{CrCl\, x\, D\, x\, Scr\, x\, 72}{K\, x\, (140 - age)} \tag{12}$$

Substitute for W_a in equation (1)

$$\therefore \mathrm{LD}_{50} = \frac{ED_{50}}{3}\, x\, \frac{CrCl\, x\, D\, x\, Scr\, x\, 72}{K\, x\, (140 - age)}\, x\, 10^{-4} \tag{13}$$

Equate equation (3) and (12)

$$\frac{TBV}{0.08} = \frac{CrCl\ x\ D\ x\ Scr\ x\ 72}{K\ x\ (140 - age)} \tag{14}$$

$$\text{TBV(K x (140–age)} = 0.08(CrCl\ x\ D\ x\ Scr\ x\ 72) \tag{15}$$

$$\text{TBV} = \frac{0.08(CrCl\ x\ D\ x\ Scr\ x\ 72)}{K\ x\ (140 - age)} \tag{16}$$

$$\text{But D} = \frac{Pcr}{Scr}\ x\ 144 \tag{17}$$

Substitute for D in equation (14)

$$\text{TBV} = \frac{0.08\left(CrCl\ x\ \left(\frac{Pcr}{Scr}\ x\ 144\right)\ x\ Scr\ x\ 72\right)}{K\ x\ (140 - age)} \tag{18}$$

Equate equation (10) with equation (13)

$$\text{LD}_{50} = \frac{ED_{50}}{3}\ \text{x}\ \left(PV + \frac{Hb}{0.33}\right)\ x\ 8\ x\ 10^{-2} = \frac{ED_{50}}{3}\ x\ \frac{CrCl\ x\ D\ x\ Scr\ x\ 72}{K\ x\ (140 - age)}\ x\ 10^{-4} \tag{19}$$

$$\left(PV + \frac{Hb}{0.33}\right)\ x\ 8\ x\ 10^{-2} = \frac{CrCl\ x\ D\ x\ Scr\ x\ 72}{K\ x\ (140 - age)}\ x\ 10^{-4} \tag{20}$$

$$\left(PV + \frac{Hb}{0.33}\right) = \frac{CrCl\ x\ D\ x\ Scr\ x\ 72}{K\ x\ (140 - age)}\ x\ 12.5 \tag{21}$$

$$\text{PV} = \left(\frac{CrCl\ x\ D\ x\ Scr\ x\ 72}{K\ x\ (140 - age)}\right)\ x\ 12.5 - \left(\frac{Hb}{0.33}\right) \tag{22}$$

$$\left(\frac{Hb}{0.33}\right) = PV - \left(\frac{CrCl\ x\ D\ x\ Scr\ x\ 72}{K\ x\ (140 - age)}\right)\ x\ 12.5 \tag{23}$$

$$\text{Hb} = PV - \left(\frac{CrCl\ x\ D\ x\ Scr\ x\ 72}{K\ x\ (140 - age)}\right)\ x\ 12.5\ x\ \frac{1}{0.33} \tag{24}$$

$$\text{Amount of Required Donor Blood} = \text{Required Recipient Wt(kg)} \times 80\text{ml/kg} \times \frac{Desired\ PCV - Recipient\ PCV}{Donor\ PCV} \tag{25}$$

TBV = Total blood volume; PV = Plasma volume; EV = Erythrocyte volume; Hb = Hemoglobin; CrCl = Creatinine clearance; D = Depuration; K = Constant (male = 1.0, female = 0.85); Scr = Serum creatinine; Pcr = Plasma creatinine; PCV = Packed cell volume.

4.5 Apitoxicosis

Honeybee is a social insect that feeds on nectar of plant and serves as source of beneficial and harmful effects (**Table 2**) which include Parkinsonism, allergy, brachial plexitis, intravascular coagulopathy, rhabdomyolysis, acute renal failure, nephrotic syndrome, anaphylaxis, fatality, acute ischemic stroke, corneal decomposition, cataract, glaucoma, seizure, ischemic attack, heart attack and acute axonal polyneuropathy [38–50]. *Apis mellifera* constitutes a significant problem of medical importance. Other important medical subspecies are *A. mellifera carnica*, *A. mellifera ligustica and A. mellifera scutellata* [13]. In India, honeybee sting affects

human beings of all ages. But cases of honeybee stings affect human between 20 and 29 years of age in Brazil [14]. However, bee envenomation is a problem in India, China, Latin America, Middle East, North and South Africa [51] and should be listed by WHO as neglected tropical disease. The venom contains alkaloids, terpenes, histamine, formic acid [52], apamin and melittin that meditate their actions via ion channels and intracellular calcium of heart cells [53] causing myocardial infarction and cardiac arrest. The venom also contains heptanone, a local anesthetic [54], which causes nausea, vomiting, diarrhea, headache, vertigo, CNS depression, incoordination, cardiorespiratory failure and hypotension [55–58]. However, *A. mellifera* venom also contains phospholipase A_2, hyaluronidase, adrenaline and serotonin, therefore causing allergic reaction, edema, skin inflammation, headache, weakness and dizziness [59] which is treated using serum [60]. But apamine, secapine, pamine, minimine, adolapine, procamine A&B, protease inhibitor, tertiapine, cardiopep, phosphatase, α-glucosidase, phospholipase B, dopamine, noradrenaline, aminobutyric acid, α-amino acids, and complex esthers produced by honeybees are used in treating a myriad of diseases [61]. Postmortem findings caused by honeybee envenomation are edema of brain, larynx, trachea as well as congestion of all other internal organs. The cause of death may be due to anaphylactic reaction [62]. The honeybee envenomation could also lead to focal neurological deficit 5 hrs post multiple stings causing acute ischemic stroke [63] and acute renal failure [64]. Immunotherapy when instituted on time is quite helpful. It involves secretion of cytokines and stimulation of T cells which indicate immune response. There is a switch from the abnormal Th_2 cytokine response to a Th_1 response by down regulation of Th_2 response or immune deviation in favor of Th_1 response, thereby producing interleukins 3–5 leading to IgE synthesis activating eosinophils and mast cells. The overall effects are venom neutralization or immunization [39].

4.6 Calculation of lethal dose of honeybee antivenom

Development of a new formula for calculation of LD_{50} of honeybee venom and antivenom is as follow:

i. If one (1) sting produced one (1) dose of venom,

ii. 1000 stings would produce 1000 x dose per sting

Therefore; NS = No of stings.

DS = Dose per sting

iii. Total Dose (TD) = NS x DS = Toxic dose

If the toxic dose (TD) can kill one hundred mice, how much can kill 50 mice?

Hence TD $\longrightarrow$ 100 mice.

$LD_{50} \longrightarrow$ 50 mice

iv. $TD = \frac{LD_{50}\ x\ 100}{50}$

$TD = 2LD_{50} = 2MLD$.

TD = Dose that kills all the mice and may not be specific because of the factors (age, sex, potency of toxin etc.) that determine toxicity level of a toxin.

v. Also TD = NS x DS = 2 x MLD

vi. Therefore, MLD = $\frac{NS\ x\ DS}{2} = LD_{50}$

Bee venom may be as poisonous as scorpion venom. Therefore, the LD_{50} formula for scorpion envenomation is applied.

vii. $LD_{50} = ED_{50}^{1/3}\ x\ Wa\ x\ 10^{-4}$ (formula for calculation of scorpion venom) [56].

Equate vi and vii.

$LD_{50} = \frac{NS\ x\ DS}{2} = \frac{ED_{50}^{1/3} x\ Wa\ x\ 10^{-4}}{1.}$

$2(ED_{50}{}^{1/3}\ x\ Wa\ x\ 10^{-4}) = NS\ x\ DS$

viii. $NS = \frac{2\left(ED_{50}^{1/3}\ x\ Wa\ x\ 10^{-4}\right)}{DS}$

ix. $DS = \frac{2\left(ED_{50}^{1/3}\ x\ Wa\ x\ 10^{-4}\right)}{NS}$

x. Lethal time 50 (LT_{50}) = $\frac{LD_{50}}{D^p}$ whereas D = dose of toxin; p = power law exponent ($^1/_3$).

xi. Potency of antivenom (PA) = Total venom used – $1EC_{50}/EC_{50}$

$1EC_{50}$ = One effective concentration fifty; EC_{50} = Effective concentration fifty.

xii. Honeybee can only sting once and dies because its sting and the venom apparatus are avulsed from its abdomen, unlike bumble bee that can sting several times as its sting is not barbed.

4.7 Application of the formula

Q1.An adult man who weighed 60 kg received 1000 stings from honey bees at the rate of 0.3 mg per sting.

i. Calculate LD_{50} and ED_{50} of the honeybees venom and the likely antivenom that should have been used prior to the man's death.

Q2. A child weighed 10 kg and received 94 stings, but eventually died before antivenom therapy.

i. Calculate the dose of venom received by the child on the condition of 0.3 mg per sting

ii. Calculate LD_{50} and ED_{50} of the venom and antivenom, respectively.

Solution 1;

Total dose (TD) of venom = Number of stings x Dose per sting

TD = 1000 x 0.3 mg = 300 mg

TD = $2LD_{50}$

$LD_{50} = \frac{TD}{2} = \frac{300}{2} = 150mg/60kg$

But if 150 mg $\longrightarrow$ 60 kg

Xmg $\longrightarrow$ 1 kg

$X = \frac{150\ x\ 1}{60} = 2.5mg/kg = LD_{50}$

But $LD_{50} = {ED_{50}}^{1/3} \times Wa \times 10^{-4}$

$2.5 = {ED_{50}}^{1/3} \times 60 \times 10^{-4}$

${ED_{50}}^{1/3} = \frac{2.5}{60\ x\ 10^{-4}} = \frac{2.5\ x\ 10^{4}}{60}$

$= \frac{2.5\ x\ 10^{3}}{6} = \frac{2500}{6} = 416.66$

$ED_{50} = \sqrt[3]{416.66} = 7.5 mg/kg$

$ED_{50} = 7.5$ mg/kg

Solution 2:

TD = NS x DS

TD = 94 x 0.3 = 28.2 mg

$TD = 2LD_{50}$

$28.2 = 2LD_{50}$

$LD_{50} = \frac{28.2}{2} = \frac{14.1 mg}{10 kg}$

$LD_{50} = 1.41$ mg/kg

But $LD_{50} = {ED_{50}}^{1/3} \times Wa \times 10^{-4}$

$1.41 = {ED_{50}}^{1/3} \times 10 \times 10^{-4}$

${ED_{50}}^{1/3} = \frac{1.41}{10\ x\ 10^{-4}} = \frac{1.41}{10^{-3}} = \frac{1.41\ x\ 10^{3}}{1}$

${ED_{50}}^{1/3} = 1410$

$ED_{50} = \sqrt[3]{1410} = 11.2 mg/kg$

Calculation of lethal time

Adult

Lethal time $(LT_{50}) = \frac{LD_{50}}{D^{p}}$

$= \frac{2.5}{0.3^{\frac{1}{3}}} = 3.72$ hr.

Child

Lethal time $(LT_{50}) = \frac{LD_{50}}{D^{p}}$

$= \frac{1.41}{0.3^{\frac{1}{3}}} = 2.10$ hr.

The weight of child and adult human, the number of honeybee stings, total dose of venom from stings, LD_{50}, LT_{50} and ED_{50} of honeybee venom and antivenom are presented in **Table 1**.

4.8 Toxic principles of honeybees

Honeybee venom contains phosphohpase A_2, melittins and antigen is found in swasp venom. The two venoms contain hyaluronidase making patients allergic to wasp venom and rarely allergic to bee venom [39]. However, 2 heptanone secreted from mandibular glands of honeybee could serve as pheromone and local anesthetic [54]. Some toxins such as saxitoxin, piratoxin-1, batrachotoxin and grayanotoxin, are specific fast sodium (Na^+) channel blockers. Whereas charybdotoxin, apamin, dendrotoxin and gaboon viper venom are specific for different types of potassium (K^+) channels. Some toxins activate L-type calcium (Ca^{2+}) and Na^+ channels in heart muscle, w-conotoxin blocks the L-type and N-type Ca^{2+} channels in neurons and maitotoxin activates voltage-independent Ca^{2+} channel in heart cells [53].

4.9 Signs of apitoxicosis

Signs of honeybee envenomation include intense local pain, erythema, 1 cm diameter of edema, local and generalized allergic reactions [39], pruritus, urticaria, facial or generalized angioedema, sense of impending doom, dyspnea due to laryngeal edema, or asthma, hypotension, light-headedness, giddiness, fainting, abdominal pain, incontinence, chest pain, visual disturbance and loss of consciousness which may happen within 10 minutes of sting [39]. Honeybee venom is highly

vasoactive, inflammatory and thrombogenic. Multiple stings could cause multiple organ dysfunctions called Kounis syndrome. The treatment is by steroid, antihistaminic and angioplasty to left anterior descending artery [49]. Occasional death is due to anaphylactoid shock [62]. Generally, the venom of wringed hymenoptera include acetylcholine, dopamine, histamine, norepinephrine, serotonin, polypeptides (protein toxins) such as apamin and melittin. Death could be due to dysfunction of immune system in which venom allergen reacts with cell bound immunoglobulin E [65].

The circulatory, dermal, respiratory and gastrointestinal systems react to sting after one or more sensitizing stings (Type 1 hypersensitivity reaction), 58% die in 6 hours [65]. Autopsy revealed that 75% of dead patients showed obstruction of airway. LD_{50} of the venom is caused by 19 stings which should be removed. Bee venom being relatively acidic should be neutralized by base. Cold pack should be applied locally to reduce pain [62]. Bee sting could cause immediate or delayed hypersensitivity reaction, leading to motor-predominant axonal polyneuropathy associated with acute inflammatory demyelination. Intravenous immunoglobulin was effective [66]. Bee sting causes increased creatinine, interstial eosinophilia, nephritis, acute tubular necrosis, anuria, decreased creatinine kinase, altered sensorium, leukocytosis and doll's eye [43]. The reported median lethal dose (LD_{50}) of bee venom is 2.8 mg/kg [61] making it extremely toxic [67]. Bee and wasp could cause acute ischemic neurological deficits 5 hours after multiple stings. Stings of 100–200 bees could be responsible for stroke [63]. Vomition, diarrhea, dyspnea, myocardial infarction, and cerebral infarction are rare [68]. Seizure, hemiparesis, aphasia, apraxia, ataxia, dysphagia and coma are also associated with wasp and bee stings [69]. Cerebral ischemia could be secondary to biogenous amine, adrenaline, platelete aggregation, thromboxane and leucotrienes [70, 71], which may lead to disseminated intravascular coagulation and death caused by 25–30 stings [41]. Parkinsonism, an extrapyramidal abnormality could be caused by an immune-mediated delayed hypersensitivity which responds to aggressive treatment using immunosuppressants [38]. Brachial plexitis, rhabdomyolysis with generalized body and muscle pain, acute renal insufficiency and liver dysfunction characterized by alkaline enuresis are signs of apitoxicosis [42]. Nephrotic syndrome secondary to hypersensitive reaction which disappeared after corticosteroid treatment (prednisolone 2 mg/kg) that reappeared after one year, hypoproteinemia, proteinuria [44], acute renal failure and cardiac arrest were also observed [45].

4.10 Diagnosis of apitoxicosis

Diagnosis is by detection of venom-specific 1gE antibodies, CAP-RAST and radio adsorbent test [39]. Intravenous administration of 125 mg of *Vipera aspis* venom was redistributed in 7 hours and neutralized by antibody 15 min after antivenom injection. Lower doses of antivenom failed to yield complete neutralization. $F(ab')_2$ and Fab could be used as antidotes [72]. Multiple organ failure and acute renal failure could be caused by wasp sting, acute tubular necrosis secondary to hemolysis; rhabdomyolysis and thrombotic microangiopathy have also been reported [46]. With supportive care, victims should survive hundreds of wasps or approximately 1000 honeybee stings [73]. Early renal biopsy is vital for patients who do not respond to supportive measures. Hence timely dialysis and steroid may improve survivability [74]. Atropine sulphate, dexamethasone, neuroxine-B, multivitamin and electrolyte therapy led to recovery of passive respiration [75]. Similar treatment may be effective in human. $F(ab)_2$- based antivenom raised in horse may lead to production of specific high 1gC titer, counter hemolysis, myotoxicity and cytotoxicity [76]. Polyclonal antibodies against *Apis indica* reversed a lot of toxic

effects in experimental mice [77], hence may be tried in human. Acute renal failure without hemolysis could be treated successfully using low dose of dopamine, fluid therapy and hemodialyis [64]. Apitoxin 1 (77.8%) and apitoxin 2 (51.9%) with LD_{50} (71.5 μg/ml) and LD_{50} (191.6 μg/ml) respectively are affected by moisture and protein content [78]. Lethal dose of snake venom could be neutralized by antidote equal to one-third of lethal dose with reference to body weight of the affected animal and safety of 10^{-4} [35]. Treatment of scorpion envenomation requires antivenom that is 66.6% of median lethal dose of scorpion venom [26]. In both cases treatments shall continue until the patients recover from the toxicity. Since kidney is affected, creatinine clearance, volume of urine, creatinine, plasma creatinine, serum creatinine and urine volume should be determined to assess the extent of renal damage, using the formulas reported by Saganuwan et al. [37]. Level of hemolysis should be assessed and quantity of hematonics and plasma expanders should be calculated. *Abrus pracatorius* could be used as hematonic [36]. Since the toxins cause neurological signs, their atomic weight may be less than 600 daltons. Hence the transport of the toxin to the brain may be by protonation or deprotonation or damage to meninges [79].

4.11 Treatment of apitoxicosis

Pheniramine maleate (1 mg/kg) and methyl prednisolone (1 mg/kg) in 100 cc physiologic serum should be administered parenterally. A loading dose of phenytoin administered against convulsion, supply of oxygen, adrenaline (0.5 mg i.m.) repeated at 10–15 min intervals, diphenhydramine, prednisolone and aminophylline for bronchospasm are highly beneficial [80]. Treatment is by administration of antihistamine for several days, steroids, chlorpheniramine, hydrocortisone, adrenaline, and inhaled B_2 agonist [81]. Bee sting could cause polyneuritis, Parkinsonism, encephalitis, acute disseminated encephalomyelitis, Guillam-Barre Syndrome, Glasgow Coma Score (GCS) of 11, myocardial infarction, pulmonanary edema, hemorrhage, hemolytic anemia, renal diseases and tonic–clonic seizure [48]. Climatic seasonal and feeding factors could qualitatively or quantitatively affect the potency of melittin and phosphohpase A2 of *Apis mellifera* [60]. The similarity in the calculated LD_{50} (2.5 mg/kg) of honeybee venom in the present study as compared to the reported value (2.8 mg/kg) in human [82] shows that honeybee venom is very toxic. The fact that 7.5 mg/kg ($3LD_{50}$) of the honeybee antivenom could neutralize 2.5 mg/kg of the venom agrees with the report indicating that antivenom of 3 folds venom LD_{50} could neutralize honey bee venom. Our findings are corroborated by the report that the dose of apilic antivenom is determined by number of stings. The higher the number of stings, the higher the dose of apilic antivenom is used [83]. The F $(ab)_2$ – based antivenom contain 1gG that prevented hemolysis, cytotoxicity and myotoxicity [84]. However, polyclonal antibody against honeybee envenomation could neutralize 40% of LD_{50} venom [76]. Venom produced by bees (apitoxin) is of two types; type I has LD_{50} of 71.5 microgram/ml and type II has LD_{50} of 191.6 microgram/mL, respectively [77] and 0.5 mg of *A. mellifera* venom can severely damage kidney of rabbit, signifying that mechanisms of lethality in animals may involve kidney failure. Change in living conditions of honeybee can lead to change in composition of their toxins [78]. The calculated LD_{50} (1.41 mg/kg) for child that weighed 10 kg in the present study agrees with the report that children, elderly and underweight people can be affected and a multiple stings of more than 500 can yield large quantity of the venom [85] which can produce more severe reactions in allergic patients [19]. Relatively higher ED_{50} (11.2 mg/kg) for the honeybee antivenom shows that the children are severely affected and as such require relatively large volume of the antivenom for

neutralization of the venom. In the present study about 7.9 LD_{50} of antivenom is required in children of 10 kg. The calculated LT_{50} of 2.10 and 3.72 hr for child and adult human respectively, agree with the report indicating that honeybee venom from over 50 stings can kill within short period of time [86]. The fact that 200 stings could cause envenoming syndrome in adult with type 2 diabetes and prostate cancer [87] and non-diseased human [83] shows the newly derived formula may be used for determination of effective dose of honeybee antivenin for all the population segments. The calculated LD_{50} (2.5 mg/kg) for honeybee venom is comparable to that of scorpion (*Mesobuthus eupeus*) venom (0.18–4.5 mg/kg). This indicates that, there is relationship between the two venoms. Majority of deaths is caused by airway obstruction and anaphylactic shock [62] and 58% of the affected humans can die in less than 1 hr and over 75% can die in less than 6 hr [65]. However, stings of 600–1500 can be survived depending on therapeutic intervention [88]. Therefore, the preliminary efficacy and safety of Africanized honeybee antivenin predicts its clinical validity [83]. Dentar bee stung patient may require keratoplasty and removal of cataract and glaucoma which may require antibiotic /steroid. Moxifloxacin (0.5%) or tobramycin (0.3%) with dexamethasone (0.1%) 6 times daily for up to 4–6 weeks are highly beneficial. Oral prednisolone (40–50 mg) may be used in place of dexamethasome [89].

4.12 Medical uses of honey

The use of raw honey, royal jelly, pollen, propolis, bee venom and wax for treatment of various medical conditions is known as apitherapy. Conditions usually treated are arthritis, multiple sclerosis, and skin diseases among others. Melittin (40–50%) is the major component of honey used as antiviral, antimicrobial, and anti-inflammatory as apamin (2–3%) increases production of cortisol in adrenal gland, adolapine (0.5–1%) is anti-inflammatory and analgesic, acting via cyclooxygenase. Nevertheless histamine (0.5–2%) cause pain and swelling, dopamine (0.2–1%), serotonin (0.5–1%) and norepinephrine (0.1–0.5%) are all neurotransmitters for mood balance. Hyaluronidase (1–2%), and phosphohpase A2 (10–12%) are enzymes for activation of immune cells, production of immunoglobulin E (1 g E) and degranulation of mast cells. The sting and bite cause fluid exudation and protease inhibitor (0.1–0.8%) act as anti-inflammatory and antihemorrhagic [89].

4.13 Spider envenomation

Eggs of black widow spider (Latrodectus), family (Theridiidae) are toxic to mammals. Species of spiders belonging to Agelenidae, Tetragnathidae, Pimoidae and Linyphiidae are also toxic [90]. Brazilian spider (*Sicarius ornatus*, Araneae, Scicariidae) venom contains active sphingomyelinase D that causes hemolysis and keratinocyte death similar to the South American loxosceles species [91]. Methyl ketones (2 –tridecanone, 2-undecanone) from tomato (*Lycopersicon hirsutum f. glabratum* are lethal to insects [92]. Black widow spiderling extract caused apoptosis and death of HeLa cell lines [93]. Spider venoms contain toxic proteins and peptides in varying compositions. Sphingomyelinase causes varying degree of dermonecrosis from American *Sicarius loxosceles* and African Sicarius [94]. However, scorpion, spider and wasp could be controlled using pesticide [95]. Spiders such as Callobius (Amaurobiidae), Antrodiaetus (Antrodiaetidae), yellow sac spider (*Cheiracanthium mildei*), erb-weaver of the genus Araneus and hobo spider bites, result in pain, redness of stung sites and muscle twitching which disappear in 12 hr [96]. Pesticides confidor and Buctril-M are used against agrobiont spiders, *Lycosa terrestris* Butt and *Oxyopes javanus* [97]. New Zealand spiders, *Latrodectus katipo* and *L. atritus* are

being threatened [98]. Latrotoxin though unique to black widow spider can be transferred to other spiders and bacteria via symbiosis. Latrodectin venom genes originate from ecdysozoan ion transport peptide (ITP) and crustacean hyperglycemic hormone (CHH) neuropeptide super family. The lower presence of latrotoxins in house spiders relative to black widow spiders, in the absence of a vertebrate alpha-latrotoxin in the house spider genome account for high potency of black widow venom [99]. Latrodectism caused by *Latrodectus spp* is manifested by local, regional or generalized pain, associated with non-specific symptoms and autonomic effects. Loxoscelism caused by *Loxosceles spp* has continuous manifestation characterized by pain and erythema that can develop into a necrotic ulcer. Systemic loxoscelism is characterized by intravascular hemolysis and renal failure. *Atran spp*, *Hadronyche spp* and *Phoneutria spp* from Brazil are also harmful. Antivenoms have been less successful in the treatment of their toxicoses [100].

4.14 Treatment of spider envenomation

Extracts of *Capsicum chinense*, *C. frutescence*, *C. baccatum*, *C. annuum* and *C. pubescens* could be used to repel spiders. *Solanum habrochaites* has methyl ketones (2-undecanone; 2-dodecanone; 2-tridecanone; 2-pentadecanone) with high acaricidal potential and may be used in control of harmful spiders. *Cheiracanthium punctorium* caused mild necrosis and dermonecrosis (necrotic arachnidism) [73]. Venoms of *Tityus serrulatus* (scorpion) and *Loxosceles gaucho* (spider) including *Apis mellifera* are highly toxic [101]. Atracotoxins targeting calcium channels blockers should be considered as conventional pesticides [102]. Sulfur induces release of cortisol from adrenal glands that protects the body from infection. Bee venoms could cause heart failure and suffocation [82]. Imidaclopid (insecticide) causes delayed and time-cumulative toxicity to bees, ants and termites [84].

5. Conclusion

The toxicity of snake and scorpion venom is dependent on the quantity of the venom, whereas apitoxicosis in human is dependent on the number of stings and dose of venom produced per sting. Snake and scorpion venoms are the most dangerous. However, venoms of some snakes and scorpions are equipotent and require 2 or more vials of antivenoms. LD_{50} of honeybee venom in adult man is 2.5 mg/kg which can be neutralized by 7.5 mg/kg ($3LD_{50}$) of antivenin. LD_{50} of honeybee venom for child is 1.41 mg/kg and can be neutralized by 11.2 mg/kg ($7.9LD_{50}$) antivenin. The venom can kill in less than 4 hr. Hence, children are more sensitive to honeybee toxicity than the adults are, and so may require higher dose of antivenom. Spider and wasp envenomation are less severe and could be treated symptomatically. All the organ systems could be affected and complications could follow multiple attacks. Hence treatment is by administration of antivenins, antiiflammatory, analgesic and respiratory support. Neurological and cardiorespiratory signs may be considered as indices of therapeutic success or failure. Prompt therapeutic intervention and hospitalization of 1 or more days could either delay or avert death.In the cases of severe anemia, blood transfusion and fluid therapy may be evident.

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