Evaluation of anti-scorpion (*Hottentota tamulus*) venom potential of native plants extracts using mice model

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**ABSTRACT**

*Hottentota tamulus* (Fabricius, 1798) (Scorpiones: Buthidae) is among the most common venomous species of scorpions in Punjab, Pakistan. The venom of this species has high risk of morbidity and mortality. Several medicinal plants have been reported to have anti-scorpion venom potential. However, there is scarcity of scientific evidences that could prove the anti-scorpion venom potential of plant extracts. The aim of present study was to evaluate the anti-venom activity of four native plants extracts, i.e. *Mangifera indica* L., *Achyranthes aspera* L., *Allium cepa* L. and *Ginkgo biloba* L. using Swiss albino mice as model. The Methanolic/aqueous extracts of plants were mixed with LD99 of *Hottentota tamulus* and injected intraperitoneally. The response was recorded till 7th day of treatment. Moreover, hematological and serological analyses were performed after administration of LD50 and LD99 doses of venom. The extracts of *A. aspera* and *A. cepa* completely neutralized (100%) the effect of scorpion venom, but neutralization effect of *G. biloba* was comparatively less (83.3%). However, *M. indica* (leaves and flowers) did not neutralize the effect scorpion venom. The extracts of plants were analyzed by GC-MS for the compositional analysis. The major compounds found in *A. aspera*, *A. cepa* and *G. biloba* were Oleanolic acid, cyclopropane, Lupeol acetate, a cholesterol, palmitic acid and Stearic acid respectively. It is concluded that *A. aspera* and *A. cepa* completely neutralize the effect of venom and could be used in future to design anti-scorpion venom agent.

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**Introduction**

Scorpion venom contains neurotoxins that can interrupt physiological activities of host by targeting the excitable neurons (Quintero-Hernández et al. 2013, Quintero-Hernández et al. 2013, Ding et al. 2014, Zhao et al. 2016, Al-Asmari et al. 2017). Some scorpion venom peptides block the potassium channels resulting in a prolonged action potential while other peptides cause the delay in inactivation of voltage gated sodium channel (Strong et al. 2001, Bosmans and Tytgat 2007). Most scorpion stings cause severe pain which runs from the site of sting to other parts of body, leading toward the rise in blood pressure, mild sweating, transient bradycardia and warm extremities (Chippaux 2012, Ahmadi et al. 2020). Other symptoms include vomiting, salivation, pulmonary edema, several neurological manifestations, hemodynamics and abnormal electrocardiography (Bawaskar and Bawaskar, 2012). Harmful effects of venom on myocardium and adrenal secretions of catecholamine have also been reported (Agrawal et al. 2015, Ahmadi et al. 2020).

Different treatments such as anti-venom, prazosin, inotropics, atropine, vasodilators and benzodiazepines are used to treat scorpion sting clinically (Bouaziz et al. 2008, Boyer et al. 2009, Bawaskar and Bawaskar 2011). Prazosin is a very potent adrenergic antagonist which lowers the blood pressure and peripheral resistance by vasodilating the peripheral vessels (veins and arteries), without raising the heart rate and disturbing the functions of sympathetic nervous system (Cohen 1970, Hess 1975, Reynolds 1982, Goodman 1996). The effect of vasodilation not only has relaxant effect on blockade of post synaptic but also on the vascular smooth muscles (Arky 1996). Prazosin inhibits the cyclic nucleotide phosphodiesterases.16. The outcome of prazosin may include the rise of...
in intracellular cyclic AMP at vascular points and cyclic GMP level at cholinergic receptor sites of heart (Hess 1975).

Other than these, various scorpion anti-venom sera have also been manufactured for the treatment of scorpion stings (Laustsen et al. 2016). Scorpion anti-venom (SAV) is the particular cure for scorpion sting but its administration still remains debatable due to its absolute specificity and economic problems (Bahekar et al. 2012, Isbister and Bawaskar 2014, Pucca et al. 2019). The composition of scorpion venom varies with species and geographical region; this limits the use of particular drug against scorpion stings (Kankonkar et al. 1998, Bawaskar and Bawaskar 2012). Hence, the cure with clinical medications currently available in market is symptomatic and nonspecific with less success.

Herbal medicines are being used since ancient times as a remedy for diseases and are preferred due to better therapeutic value, fewer adverse effects, abundance, availability, affordability, effectiveness and link with cultural traditions (Ansari and Farha Islam 2012, Aziz et al. 2018, Shah 2018, Tounekti et al. 2019). These medicines containing diverse array of constituents are gaining popularity as a natural treatment of several disorders (Yadav et al. 2011, Maiti et al. 2017). Herbal drugs are used for basic health care by almost 80% of the world’s population (Ekor 2014). Furthermore, many indigenous communities use herbal medicines for treatment and management of scorpion sting (Singh et al. 2012, Kale et al. 2013). However, to date, the treatment of scorpion sting by different herbal medicines is not fully accepted by modern physicians (Kale et al. 2012).

Many plant-based medicines have been manufactured by using traditional knowledge. Thus, protecting, conserving and practicing (if scientifically proved) traditional knowledge is of noble importance (DeFilippis and Krupnick 2018). A wide variety of medicinal plants exist in Pakistan but there is dire need to scientifically evaluate their anti-scorpion venom activities (Qureshi et al. 2010, Nasim et al. 2013). Different important healing agents for various fatal diseases can be discovered by scientific studies of these native plants (Nasim et al. 2013). Previously, Achyranthes aspera is used as antimicrobial, immunostimulant, anti-inflammatory, hypoglycemic, anti-oxidant, diuretic, antihypertensive, cardiac stimulant, analgesic, hepatoprotective and antifertility agent (Dey 2011). Mangifera indica possesses anti-inflammatory, anti-viral, anti-diabetic, anti-oxidant, immunomodulation, anti-microbial, hepato protective and gastro protective properties (Parvez 2016). Allium cepa bulb contains anti-inflammatory, anti-carcinogenic, anti-diabetic, anti-septic, diuretic, hypotensive and hypoglycemic properties (Kumar et al. 2010). Ginkgo biloba has been reported to reduce platelets aggregation, oxidative stress, neural damages and act against inflammation, tumor and aging (Chan et al. 2007).

These plants have been used traditionally by native population against scorpion venom. The roots of A. aspera are mixed with drinking water and given orally once a day to neutralize the effect of scorpion venom (Kunjam et al. 2013). Application of paste of ground leaves of A. aspera on sting site for 1 h and using fresh juice of leaves or fresh bulbs of A. cepa (without using any solvent) on sting site can provide the patient with relief on sting site; also the use of 2–3 spoons of dried powder of leaves of M. indica daily can cure the scorpion sting (Butt et al. 2015). In past, the leaves of G. biloba were used against scorpion venom which results in protease inhibitory or anti-oxidative effect (Dey et al. 2013).

In the present study, we have selected four native plants, i.e. A. aspera (prickly chaff flower), A. cepa (onion), G. biloba (maiden hair) and M. indica (mango) from Pakistan. These plants were selected because they have been used traditionally as an anti-dote against venom but no clear scientific verifiable evidence have been reported. The aim of this study was to evaluate the anti-scorpion venom potential of four native plants extracts using animal model, i.e. Albino mice. Basically, this study has two major parts (1) to check the toxicity of venom, (2) to neutralize the toxicity of venom using plant extracts.

Materials and methods

Ethical approval

The study was conducted after the ethical approval of Institutional Bioethics Committee (Figure S1).

Collection of scorpions and their maintenance

The study was conducted from May 2018 to August 2019 at Department of Zoology, Government College University (GCU) Lahore. Hottentotta tamulus (n = 30) scorpions were sampled from the sandy areas of Sargodha (31°55'59"N, 72°54'22"E; 32°06'74"N, 72°52'38"E) and Mianwali (32°02'07"N, 72°55'48"E; 32°38'77"N, 71°31'69"E) districts of Punjab, Pakistan. Ultra violet (UV) lights were used to collect scorpions at night. The collected scorpions were transferred to the laboratory, placed in small transparent plastic
containers (17 × 9 × 9 cm) (L × W × H) containing 2–3 inches base of sand, pores for ventilation, ambient temperature and small water receptacles (Yaqoob et al. 2016). Scorpions were fed with houseflies, grass hoppers, cockroaches, crickets once a week (i.e. one big grasshopper/cockroach/cricket or 5 house flies for each scorpion). As, they were nocturnal they were placed away from direct sunlight.

**Venom extraction**

Venom was extracted by electrically stimulating the tail of scorpion. For this, two to three brief electric shocks (25 V) were administered with electrodes by carefully fixing the scorpion onto the even surface of petri plate with scotch tape (Yaqoob et al. 2016). For good conductance of electric current, scorpion’s tail was dripped by 2–3 drops of 10% saline solution. Then, the venom was harvested in the blue tip through capillary action, transferred to eppendorf and stored at −20°C for further use. During the venom extraction scorpions were not anesthetized.

**Calculation of lethal dose (LD$_{50}$ and LD$_{99}$)**

To determine volume of venom, it was collected in graduated capillary tube. The collected venom was mixed with distilled water in the ratio of 1:2 (venom: distilled water). The diluted venom was then subjected to lyophilization for accurate calculation of lethal dose (LD$_{99}$). Venom was lyophilized by using lyophilizer. The lyophilized venom was stored at −20°C for further use.

A 20 mg/ml stock solution of venom in double distilled water was prepared and different doses of venom were intraperitoneally injected to record its toxic/lethal effects on mice model as detailed in Table S1.

**Study animals**

A total of 110 healthy Swiss Abino mice (Mus musculus) of both sex, of average weight 25–30 g and 6–8 weeks old were used in this study. All mice were housed in polypropylene transparent cages under controlled light, humidity (40–50%) and temperature (26–32°C). They were fed with 13% protein diet (5 g/ mice) daily with continuous supply of water.

**Hematological and serological analyses**

To study the hematological and serological effect of the H. tamulus venom on the blood and serum, a total of 18 healthy Swiss Albino mice were taken and divided into three groups containing 6 animals each. Group 2 and 3 were administered with LD$_{50}$ and LD$_{99}$ while Group 1 was taken as control. After 30 min of dose administration, cardiac puncture method was used to collect the whole blood (1–1.5 ml) in EDTA tubes to avoid coagulation for further processing. CBC and Serum electrolytes test were performed by the Hematology Analyzer and Chemistry Analyzer BTS-350 (Biosystems) respectively at Diagnostic Laboratory of University of Veterinary and Animal Sciences (UVAS), Lahore.

**Collection of plants**

Anti-dote effect of four native plants including A. aspera (prickly chaff flower), A. cepa (onion), G. biloba (maiden hair) and M. indica (mango) was evaluated in the present study. The leaves of G. biloba and leaves and flowers of M. indica were collected from Botanical Garden GCU Lahore (31°33'30"N 74°19'44"E) while A. aspera leaves were sampled from Changa Manga forest (83 Kilometers South-West of Lahore, 31°05'N 73°58'E). Moreover, the extract of A. cepa was prepared in Entomology and Biomaterial Research laboratory, Department of Zoology, GCU Lahore (31.5732° N, 74.3079° E). Plants were submitted to herbarium for acquisition of Herbarium Identification (ID) numbers. Details of Herbarium IDs are: GC. Herb. Bot. 3326 for G. biloba; GC. Herb. Bot. 3327 for A. cepa; GC. Herb. Bot. 3328 for A. aspera; GC. Herb. Bot. 3329 for M. indica.

**Preparation of plant extracts**

After sampling, flowers and leaves of G. biloba, M. indica and A. aspera were thoroughly rinsed with water and shade dried. Fine powder was made with mortar and pestle and stored in airtight jars until further use. The methanolic extracts of plants were prepared according to protocol explained by Turner (1965) (Mahanta and Mukherjee 2001, Candan et al. 2003, Mehmood et al. 2015). Dried parts of plants (40 g) were soaked in 95% methanol overnight. The mixture was then filtered, packed in thimble of soxhlet and extract was prepared by refluxing the mixture using 95% ethanol at 60–80°C. Dark green to brown in color extract was then mixed in distilled water and indirectly heated on water bath at 60°C to evaporate organic component. The remaining settled components were lyophilized and powder was then stored inside the refrigerator at 4°C.
Furthermore, *A. cepa* concentrate was extracted manually by pressing it tightly by hand and then stored at −20 °C in refrigerator. The stored extract was then lyophilized and powder was kept at 4 °C until further use.

**Gas chromatography-mass spectrometry of plant extracts**

Gas chromatography-mass spectrometry (GC-MS) is a laboratory technique that involves separation properties of gas-liquid chromatography with the feature of detection by mass-spectrometry to identify various substances within a test sample. GC-MS is used to separate the volatile substances in sample while MS refers to their identification on the basis of their mass. For GC-MS the vapor pressure of analyte should be between 30 and 300 °C. GC-MS offers the identification based on retention time matching that may be incorrect. GC-MS commonly uses chemical ionization (CI) and electron impact (EI) techniques (Chauhan *et al.* 2014). The parameters used in GC-MS analysis were Retention time (RT), I Time, F Time, Area, Area %, Height, Height %, A/H and Base m/z.

For GC-MS, powder of each plant part was liquefied in 95% ethanol overnight. After filtration, small portion of organic mixture was subjected to GC-MS for separation of volatile organic compound and extract components analysis. The aqueous extract of onion was mixed with organic solvent, kept for 4–6 h in a separating funnel and then organic layer was separated for GC-MS.

**Neutralization effect of Hottentota tamulus venom by plant extracts and prazosin**

Animals were divided into seven groups containing 6 mice each. Groups 1 and 2 were taken as positive and negative control respectively while the groups 3, 4, 5, 6 and 7 were experimental.

**Administration of dosage to control group**

Group 1 (positive control) was given Prazosin (1 mg/kg of mice) after 1 h incubation at 37 °C with LD99 of *H. tumulus* venom (Attarde and Apte 2013). Saline solution mixed with the LD99 of *H. tumulus* was injected to group 2 (negative control) animals.

**Administration of doses to experimental groups**

The doses for experimental groups were prepared according to Alam and Gomes method. Each lyophilized plant extract (1 g/kg of mice) was mixed in double distilled water and incubated at 37 °C for 1 h with LD99 of *H. tumulus*. After that mixture was centrifuged at 2000 rpm for 10 min (Alam and Gomes 2003). The supernatant was injected to each mouse of experimental groups intraperitoneally. In each group, duration of survival and number of survived animals were noted for 7 days. All the observations were taken by one person to avoid the biasness. An overview of experimental design is given in Figure 1.

**Statistical analysis**

For statistical analysis of data, one way analysis of variance (ANOVA) was applied using software SPSS. *p* Values ≤0.05 was considered as significant while *p* values ≥0.05 was considered as non-significant to avoid biasedness.

**Results**

**Calculation of lethal doses of Hottentota tamulus venom**

The value of LD99 and LD50 for mice calculated by Probit analysis was 0.013 mg/g and 0.011 mg/g respectively. A minor difference (0.002 mg/g) was found between LD99 and LD50 (Table S2).

**Hematological analysis and serum chemistry**

After injecting the doses (LD50 and LD99), number of RBCs and platelets were slightly increased. Statistical analysis showed a non-significant effect of venom on mean count of RBCs (*F*2,6 = 3.03; *p* = 0.124) and HgB (*F*2,6 = 2.42; *p* = 0.17) while the mean count of WBCs and platelets were significantly increased after administration of dose, i.e. For WBC: *F*2,6 = 16.61; *p* = 0.004; For platelets: *F*2,6 = 6.8; *p* = 0.029. Detailed comparisons are given in Table 1.

Statistically, there was non-significant effect of *H. tumulus* venom on blood Sodium (Na⁺) ions of mice, i.e. *F*2,6 = 3.0; *p* = 0.125 as depicted in Table 2. However, values of Potassium (K⁺) ions were significantly increased in experimental groups as compared to control, i.e. *F*2,6 = 58.3; *p* = < 0.001.
Toxicity of Hottentota tamulus venom and its neutralization by plants extracts

The mortality was 100% in negative control group at 24 h post treatment. However, no mortality (100% survival) was found in groups treated with mixture of LD$_{99}$ + Prazosin (+ve control), LD$_{99}$ + A. aspera and LD$_{99}$ + A. cepa. But, the survival rate in the groups treated with the mixtures of LD$_{99}$ + G. biloba, LD$_{99}$ + M. indica (leaves) and LD$_{99}$ + M. indica (inflorescence) was 83.3%, 50% and 33.3% respectively at 24 h (Table 3).

Table 1. Effects of venom on the complete blood count (CBC).

| Groups   | Mean RBC (10$^{12}$/l) | Mean WBC (10$^{9}$/l) | Mean platelets (10$^{9}$/l) | HGb (g/dl) |
|----------|------------------------|----------------------|-----------------------------|------------|
| Control  | 6.71 ± 0.39$^{\text{Ns}}$ | 2.0 ± 0.34$^{a}$     | 503 ± 50.8$^{a}$            | 12 ± 2.29$^{a}$ |
| LD$_{50}$ | 7.6 ± 0.96$^{\text{Ns}}$ | 4.6 ± 1.18$^{b}$     | 710 ± 148.8$^{b}$          | 14 ± 2.02$^{b}$ |
| LD$_{99}$ | 8.0 ± 0.26$^{\text{Ns}}$ | 5.18 ± 0.12$^{c}$    | 783 ± 57.7$^{c}$           | 15.2 ± 1.02$^{c}$ |

Note: Values in each column with different superscripts are standard deviation.
Ns: Non-significant difference; a,b,c: Significant difference. RBC: Red blood cell; WBC: White blood cell; LD: Lethal dose; HGb: Hemoglobin.
The data of mortality and survival was recorded for 7 days. It is evident from Table 3 that the survival rate of groups 6 and 7 was 50% and 33.3% respectively at 24 h which was decreased to 0% at 168 h.

**Gas chromatography-mass spectrophotometry (GC-MS)**

Several compounds were found in the plant extracts as a result of GC-MS. However, some anti-scorpion venom active components were indicated as described already in the literature (Table 4).

**Discussion**

The complete blood count (CBC) of mice treated with LD₅₀ and LD₉₀ was performed to compare the blood cells count in normal and treated groups. A significant increase in total leukocyte count (TLC) was recorded. The average TLC number was increased from 2.0 ± 0.34 (before treatment) to 5.18 ± 0.12 (after treatment). Our results were in accordance with the findings of previous reports (Chitnis et al. 2012, Murthy and Rao 2014). They have also reported an elevated number of WBC’s in the envenomed people due to inflammation, stress, and to overcome the toxicity of venom which leads toward leukocytosis. High level of WBC’s may suggest the increase in the inflammatory or cytokine reaction after the scorpion venom injection. The mean platelets count of intraperitoneally injected venom groups was also significantly increased from 503 ± 50.8 (before treatment) to 783 ± 57.7 (post treatment). Similar results were found by Emam et al. (2008), i.e. increase in platelets count by 61.4% in venom exposed organisms. The platelets help to resist the blood loss and provide an active surface for arrangements of enzyme complexes and enhance fibrin formation (Heemskerk et al. 2002). The increase in platelets count would result in the onset of pulmonary hemorrhage and thrombocytopenia (Longenecker and Longenecker 1981). Hemorrhagic abrasions were found in the lungs, kidneys and heart after scorpion sting which are in turn lead to increase in platelets count (Correa et al. 1997). Due to thrombocytopenia, platelets aggregation may occur and epithelial damage may also be caused due to the toxins in venom (Franchini et al. 2006).

In the current study, there was non-significant difference in the mean RBC’s values in venom treated and untreated control groups. Decrease in number of RBC’s were reported by Dehghani et al. (2012), Mishal et al. (2015) and Abdoon and Fatani (2009). This decrease in the level of RBC’s suggests their hemolysis. Emam et al. (2008) recorded 48.73% hemolysis of RBC’s while Pipelzadeh et al. (2007) and Jalali et al. (2012) found prominent hemolytic symptoms after administration of the venom. However, the level of hemoglobin was increased in experimental groups due to the hemolysis of erythrocytes. This elevated level of hemoglobin was also recorded by other scientists (Shahbazzadeh et al. 2009).

There was non-significant difference of Na⁺ ions in experimental and control groups. However, a marked increase was recorded in the levels of potassium ions in venom treated groups. These results revealed the specificity of H. tamulus venom for K⁺ activated channels but not for Na⁺ channels. The reason for this selectivity are the toxins (tamulotoxin and iberiotoxin) found in the venom of H. tamulus, having strong affinity with K⁺ ions but not with Na⁺ ions due to their polypeptide structures and binding properties (Quintero-Hernández et al. 2013). As a result, high Potassium levels may lead to hyperkalemia with increased release of neurotoxins which evoke autonomic storm with prolonged and persistent depolarization of nerve impulse (Bawaskar 1982, Gwee 2002, Bawaskar and Bawaskar 2012). The increased

| Groups                  | % survival at 24 h (1st day) | % survival at 72 h (3rd day) | % survival at 168 h (7th day) | Net % age survival after 7 days/total mice |
|-------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------------------|
| LD₉₀ + saline (Negative control) | 0                             | 0                            | 0                             | 0/6                                      |
| LD₉₀ + Prazosin (Positive control) | 100                           | 100                          | 100                           | 100/6                                    |
| LD₉₀ + A. aspera         | 100                           | 100                          | 100                           | 100/6                                    |
| LD₉₀ + A. cepa           | 100                           | 100                          | 100                           | 100/6                                    |
| LD₉₀ + G. biloba         | 83.3                          | 83.3                         | 83.3                          | 83.3/6                                   |
| LD₉₀ + M. indica (leaves) | 50                            | 0                            | 0                             | 0/6                                      |
| LD₉₀ + M. indica (inflorescence) | 33.3                         | 0                            | 0                             | 0/6                                      |

LD: Lethal dose; A. aspera: Acryanthes aspera; A. cepa: Allium cepa; G. biloba: Ginkgo biloba; M. indica: Mangifera indica.
potassium reflux also contributes to the extended hyperglycemia and glycolysis (Petricevich 2010). The reason behind the $K^+$ level increase might be due to the minor renal failure followed by vasoconstriction and hypertension in the liver (D’Suze et al. 2003, Kumar and Basheer 2011). Contrary to this, Strong et al. (2001) found that tamulotoxin and iberiotoxin of H. tamulus venom inhibit the potassium channels selectively which prolonged the action potential across the excitable cell membrane of potassium channels.

To neutralize the toxicity of venom four plant extracts were used. The mortality rate in negative control group (untreated) was 100%. Whereas, there was no mortality in groups treated with LD$_{99}$ mixed with A. aspera (Alam and Gome’s method). The survival rate remained 100% till 7 days post treatment. The most common components recorded in A. aspera were Oleonolic acid and Cyclopropane. The Oleonolic acid, a triterpenoid, has been reported to inhibit the activity of metalloprotease and phospholipase A2 which are major enzymes responsible for toxicity of venom and cause inflammation, pulmonary edema and hemorrhage (Gupta and Peshin 2014). Similarly, Cyclopropane, along with its Methyl ester derivatives are present in A. aspera. This component possesses anti-inflammatory, anti-myocarditis and anti-hypertensive effects (Kothapalli et al. 2016). These components might have role in neutralizing the effect of venom, but it cannot be stated with full confidence. Further, investigations are required to establish the fact.

The survival rate in the mice treated with aqueous extract of A. cepa was also 100%. A terpenoid, Lupeol

Table 4. Anti-scorpion venom active components found in plant extracts.

| Sr. no. | Plant name | Total components found | Active components against H. tamulus |
|--------|------------|------------------------|-------------------------------------|
| 1      | Achyranthes aspera | Bicyclol,5H-Cyclopropane, Hexadecanoic acid, Methyl ester, 7-Dehydrocholesteroyl isocaproate, 11-Octadecenoic acid, methyl ester and 9-Octadecenoic acid, 2-hydroxy ethyl ester | Oleonolic acid, Cyclopropane |
| 2      | Allium cepa | Lup–en–3-ol– acetate, Epoxy-7, alpha.-bromocholestan-3.beta.-ol, Isopropyl Myristate, Hexadecanoic acid, Methyl ester, silane and 7-Hexadecenoic acid, Methyl ester | Lupeol acetate, Cholesterol |
| 3      | Ginkgo biloba | Hexadecanoic acid, Methyl ester, 9,12Octadecanoic acid,Methyl ester, 11Octadecanoic acid,Methyl ester, 10 Octadecanoic acid,Methyl ester and Octadecanoic acid, Methyl ester | Stearic acid |
| 4      | Mangifera indica (leaves) | Bicyclol, Uridine, Stridecafluorohexyl, Hexadecanoic acid, Methyl ester, 6,25-tetraatriactontadien-2-one and 9-Octadecenoic acid, methyl ester | No anti-scorpion venom active component |
| 5      | Mangifera indica (inflorescence) | Butyraldehyde, Butane,1,1-diethoxy-2-methyl, Benzene, 1,2,4-trimethyl, Benzene, 1,2,4-trimethyl, 1,3,5-tri-tert-butyl-3-(4-Norcaren-2-one), Benzene,2-ethyl-1,4-dimethyl, Ethoxypropionaldehyde diethyl acetal, Benzene,4-ethyl-1,2-dimethyl, Benzene,2-ethyl-1,3-dimethyl, Benzene,2-ethyl-1,4-dimethyl, Benzene,4-ethyl-1,4-dimethyl, Benzene, 1,2,3,5-tetramethyl, Benzene, 1,2,3,5-tetramethyl, 1H-Indene or 2,3-dihydro-4-methyl, 1,3-Cyclopenta diene or 1,2,3,4-tetramethyl-5-methylene, Benzene, 1,3-dimethyl-5-(1-methylethyl), Phenol, 2,4-bis(1,1-dimethylethyl, 1-Hexadene, Diethyl Phthalate, 1-Octadecene, 2-Butenoic acid, 1-Eicosanol, Thiocyanic acid, alpha.-d-Galactopyranose, alpha.-d-Galactopyranose, 9-Octadecanamide, N,N-Dimethyldecanamide | No anti-scorpion venom active component |

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acetate that is found in onion extract exhibit neutralization effect against neurotoxicity, cardio toxicity and respiratory failures caused by venom (Tomaz et al. 2016). It also reduces lethality, edema, hemorrhage and phospholipase A2 activity induced by venom (Chatterjee et al. 2006). A Cholesterol (5-beta, 6-beta-Epoxy-7.alpha.-bromocholestan-3.beta.-ol) which is a steroidal compound is also found in onion has also been shown to neutralize the toxicity induced by venom (Torres et al. 2013).

The neutralization effect of G. biloba was comparatively less (83.3%) as compared to A. aspera and A. cepa. Fatty acids like Stearic acid and Palmitic acid are commonly found in this plant. However, the dominant one is 11-Octa-decanoic acid, Methyl ester (Stearic acid) some novel stearic acid analogues are 1,3,4-oxadiazole and 1,2,3 triazole which have anti-depressant activity. The fatty acids found in G. biloba leaves extract have been reported to be helpful to overcome the neurophysiological disorders and depression (Jubie et al. 2012). It can be predicted that G. biloba might have role of this component in neutralization of scorpion venom.

Mangifera indica (leaves and flowers) did not contribute in neutralizing the effect of scorpion venom in mice rather, it just increases the mean survival time of mice, i.e. upto 24h. But, even this survival was compromised, i.e. the live mice were physically highly inactivates, they were not taking any feed or water and have increased heart rate. The reason for no neutralization effect of venom by Mango plant may be due to pharmacokinetic or dynamic reasons. However, Sahreen et al. (2011) and Nasim et al. (2013) reported compounds like glucopyranose, pentahalloyl and phenols in Mango flowers and their anti-venom effect. Similar compounds were found in our study but, the experimental results were contrary to the previous studies. The reason for this might be the relatively prolonged observation period of 7 days than 24h because mice remained alive till 24h but they died after that. So, due to no neutralization effect it cannot be suggested to use Mango plant extracts in designing anti-venom drugs. However, none of the active compounds against scorpion venom were tested; further studies need to be done in this regard.

In previous researches on the evaluation of anti-venom potential of plant extracts different survival rates were found. Study on anti-venom activity of Aristolochia indica plant extract against scorpion venom was done by Attarde and Aptes (2013). The plant extract was given by two methods; one method is oral application of extract after LD99 injection and the other used was Alam and Gome’s method. The survival rate found in both was the same, i.e. 50% within 24h of using plant extract. But, Alam and Gome’s method showed relatively increased mean survival time (88 min) than oral application (59 min). So, Alam and Gome’s is more appropriate because giving plant extract orally need more time for it to digest and metabolize while the venom running in blood shows its toxic effects immediately within few minutes and may lead to death. The ethanolic extract of Aristolochia indica showed neutralization effect against scorpion venom due to compounds like Lactones, Aristololide, Quinonoes, Aristolindiquinone and it is claimed that Sesquiterpenes is an active component found in leaves of indica plant and have anti-venom property due to the alterations in enzymes and peptides of venom. The ethanolic extract of Andrographis paniculata didn’t show any neutralization effect against H. tamulus scorpion venom independently as the survival rate by giving the mixture of Plant extract and venom was 0%. But, the mixture of LD99 + Anti-scorpion venom showed 33.33% survival. However, the mixture of LD99 + Plant extract + Anti-scorpion venom resulted in 50% mortality which revealed that A. paniculata boosts up the activity of anti-scorpion venom (ASV), but does not have neutralization effect separately. The activity of this plant may be due to the presence of cinamic acid derivatives, nitro compounds, saponins, tannins, alkaloids, flavonoids which have a binding of proteins and enzyme inhibiting activities of venom. Moreover, andrographolide is present which modify the actions of enzymes and proteins (Kale 2013).

Conclusion

It is concluded from the present study that the adverse effects on health and physiology of living organism by scorpion sting can be neutralized by using A. aspera and A. cepa. These plants could be used in the manufacturing of anti-scorpion venom drugs in future. However, none of the active compounds found in them against scorpion venom were tested individually and need further research.

Disclosure statement

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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