Anti-venom potential of Pakistani medicinal plants: inhibition of anticoagulation activity of *Naja naja karachiensis* toxin

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Phospholipase A (PLA) is ubiquitous in nature and the most noxious component of *Naja naja karachiensis* venom. The present work was designed to study the role of PLA as anticoagulant in an egg yolk assay and to assess the potential of the extracts of 29 medicinal plants of Pakistan to counter this action. Coagulation assays with venom were performed *in vitro* with hen's egg yolk mixture. Subsequently this effect was endeavoured to neutralize with plants extract and compared with standard antisera. Venom at concentration of 5 μg clotted within 125 sec compared to venom-free egg yolk (control) mixture which clotted in 100 sec. *Citrullus colocynthis, Rubia cordifolia* and *Stenolobium stans* (11% extracts) were found to stop anticoagulant action as recorded with reference standard antidote. On the other hand, *Albizia lebbeck, Brassica nigra, Matthiloa incana, Nerium indicum* and *Rhazya stricta* (17% extracts) were declared completely abortive. Moreover, *Allium cepa, Allium sativum, Althaea officinalis, Bauhinia variegate, Calotropis procera* (flowers), *Calotropis procera* (exudates), *Cedrus deodara, Citrus limon, Cuminum cyminum, Enicostemma hyssopifolium, Fogonia cretica, Leucas capitata, Momordica charantia, Ocimum sanctum, Pinus roxburghii, Pistacia integerrima, Psoralea corylifolia, Sapindus mukorossi, Terminalia arjuna, Trichodesma indicum* and *Zingiber officinalis* (72% extracts) showed restoration against anticoagulation behaviour, i.e. anticoagulation decreased from 92% to 20%. However, further research is inevitable for isolation and structure elucidation of bioactive constituent(s) from active plants extract.

Keywords: Anti-venom, egg yolk assay, *Naja naja karachiensis*, phospholipase A.

SNAKE bite envenomation is a global occupational peril which has provided an impetus to scientists to study the phenomenon of snake poisoning[1]. Snake venom is an intricate mixture of different compounds, mainly various proteins that exhibit both enzymatic and nonenzymatic actions. Among enzymatic proteins phospholipases, proteases (both serine and metalo), hyaluronidases, oxidases, nucleotidases and phosphomonoesterases are more common. Phospholipases constitute the most important family of snake venom enzymes[2]. Owing to their ubiquitous nature they are found in both mammals and deadly poisonous snakes. Mammalian phospholipases are non-toxic and unable to induce potent pharmacological effects. However, they play a pivotal role in signal transduction, catalytic pathways, substrate specificity, remodelling of membranes by deacylation or reacylation, fertilization and hypersensitivity. On the other hand, snake venom phospholipases are poisonous components in venom or their protein complexes[3]. In the late 19th and early 20th centuries their diverse functions stemmed to classify these enzymes. According to their site of action in phospholipids they are grouped into phospholipases A_1 (hydrolyses 1-acyl group), phospholipases A_2 (hydrolyses 2-acyl or central acyl group) and phospholiapses C and D that cleave phosphodiester linkages[4–6]. On the basis of their structural features they were classified into group I (from old world snakes), group II (from New World snakes), group III (from bee venom), group IV (Ca^{++} dependent), group V (macrophage secreted) and group VI (Ca^{++} independent). However, the latest revision enunciated that phospholiapses could be clustered into secreted $sPLA_2$s, cystolic cPLA2s, calcium-independent $iPLA_2$s and lipid lipoprotein associated (LP) PLA_2s[7].

Several snakes have been reported to possess phospholipases for example, *Austrelaps superbus*, *Bothrops leucurus*, *Daboia russelli*, *Echis ocellatus*, *Lachesis muta*, *Naja naja*, *Pseudechis australis* and *Protobothrops flavoviridis*[8]. Due to snake bite envenomation (phospholipases toxicity) several pathophysiological disorders are generated like neurotoxicity, myotoxicity, inflammation, hemolysis, necrosis, amputation and anti-coagulation. Reactive oxygen and free radical generation imparts phospholipases fatality in victims of snake bite[9,10]. To combat their deleterious and detrimental effects, various phospholipase inhibitors play a major role to save the victims.

Natural antidotes (mostly medicinal plants) have been documented previously to possess several chemical constituents to inhibit snake venom action[11]. It was reported that 578 medicinal plants are active against snake venom. However, few plants were found with novel chemical constituents with a marked mechanism of action[9]. Pakistan is a hub of medicinal flora and the rural population depends on medicinal plants for health-related issues. Majority of Pakistani plants have not been scientifically evaluated for their anti-venom (antiphospholipases) potentials despite claims mentioned in folklore[11,12].

To bridge this gap in the present study medicinal plants distributed in different areas of Pakistan were collected to

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validate them scientifically against *N. n. karachiensis* venom phospholipases. This comprised of *Albizia lebbeck* (L.) Benth, *Allium cepa* L., *Allium sativum* L., *Althaea officinalis* L., *Bauhinia variegate* L., *Brassica nigra* (L. Koch), *Calotropis procera* (Wild.) R.Br, *Cedrus deodara* G. Don, *Citrullus colocynthis*, *Citrus limon* (L.). Burm. f, *Cuminum cyminum* L., *Enicostemma hyssopifolium* (Willd.) Verdoorn, *Fogonia cretica* L., *Leucas capitata* Desf, *Matthiloa incana* (L.) R.Br, *Momordica charantia* L., *Nerium indicum* Mill, *Ocimum sanctum*, *Pinus roxburghii* Sargent, *Pistacia integerrima*, *Psoralea corylifolia* L., *Rhazya stricta* Dcne, *Rubia cordifolia*, *Sapindus mukorossi* Gaertn, *Solanum xanthocarpum* Schard & Wendle, *Stenolobium stans* (L.) D. Don, *Terminalia arjuna* Wight & Arn, *Trichodesma indicum* (Linn) R.Br and *Zingiber officinalis* Rosc in comparison with standard antidote used locally in different hospitals and clinics of Pakistan.

Pakistani black cobra snakes were collected from snake of charmers Cholistan desert, Punjab province of Pakistan. Pakistani patternless cobra snakes were duly identified by zoologist.

Venom from *Naja naja karachiensis* (*naja. n. karachiensis*) was collected by pressing the glands of the snakes below their eyes and subsequently freeze-dried. After lyophilization powder venom was kept in a light-resistant and air-tight container at 2–8°C. Before use it was reconstituted in terms of dry weight[13].

Ethnobotanically claimed medicinal plants of Pakistan having anti-venom activity were collected from different locations in the country. Details about these medicinal plants are given in Table 1. After collection they were authenticated by an expert botanist, Altaf Ahmad Dasti (Institute of Pure and Applied Biology, Bahauddin-Zakariya University, Multan, Pakistan). Voucher specimens were deposited in the herbarium of the department. Various voucher numbers were allotted to different plants for discrimination.

After washing and shade-drying different plant materials were chopped and crushed to obtain bulk powder. Methanol was used as solvent for extraction. One kilogram dried powder of each plant material was soaked in 5 l of methanol in extraction bottles. Homogenates were soaked (at 25 ± 3°C) for a period of one month and filtered twice, initially by ordinary filter paper and later on by Whatman filter paper no. 41. Methanol was evaporated at room temperature. After evaporation different extracts were weighed and preserved for further use[13].

In order to compare potency of test materials (plant extracts) with standard antidote, equine antisera (manufactured by Bharat Serums and Vaccines Limited, Ambernath, India) was obtained from the pharmacy of a local hospital[14].

Assays for the confirmation of snake venom PLA and its particular class (strong or weak anticoagulant) were performed using egg yolk protein as a substrate.

Briefly, a suspension was prepared by mixing an egg yolk (9 ml), sodium chloride (2%, 2.51 ml), ethylene diamine tetra acetic acid (0.5%, 1.49 ml), calcium chloride (1%, 4.44 ml), Tris-HCl buffer (50 mM, 2 ml, pH 7.5) and isosaline (0.56 ml) in required concentration and quantity. Egg yolk suspension was mixed with the test sample of venom of *N. n. karachiensis* (25 μg/ml) and subjected to incubation at 37°C for 60 min. Incubate was shifted to boiling water bath and time required to coagulate the suspension was noted. Saline was used as control, while venom (5 μg) was used as positive control. Enzymatic activity was expressed as percentage and inhibition of enzymatic activity was recorded using pre-incubated venom with different plant extracts. Inhibition of enzymatic activity was measured by pre-treated venom (5 μg) with different plant extracts at a concentration of 5 μg/ml for 30 min[15,16].

All numerical results are mentioned as mean ± standard deviation, which were calculated using Microsoft Excel® 2007.

Anticoagulation is one of the prominent pharmacological actions induced by *N. n. karachiensis* venom. Hen's egg yolk model was selected to evaluate the slowdown of anticoagulant action on egg yolk mixture by various antidotes. Saline egg yolk mixture was used as control, which was found to congeal in 100 sec in boiling water bath. Venom for *N. n. karachiensis* (5 μg/200 μl) was found to congeal egg yolk mixture at 125 sec as compared to venom free egg yolk mixture (control) whose clotting time was recorded at 100 sec. This delay in coagulation was due to abundance of snake venom enzymes phospholipases A. Details are given in Table 2.

In order to cope with snake venom anticoagulant activity, 29 selected samples of medicinal plants were tested against phospholipase A action. Furthermore, their results were matched with standard antisera to compare satisfactory outcomes. Among plant extracts only three were found to nullify (100%) anticoagulant effect as observed with standard anti-sera (reference standard). These valuable plants are *C. colocynthis*, *R. cordifolia* and *S. stans*. Samples of *A. lebbeck*, *B. nigra*, *M. incana*, *N. indicum* and *R. stricta* were completely abrogated to prove any fruitful effect. The remaining medicinal plant extracts were found to have different tendencies (percentage inhibition) for inactivation of phospholipase A. Among these, *P. corylifolia* (92%), *Z. officinalis* (92%), *C. deodara* (80%), *L. capitata* (80%), *S. mukorossi* (80%), *A. cepa* (76%), *B. variegate* (76%), *C. procera* flowers (76%), *M. charantia* (76%), *C. limon* (64%), *Enicostemma hyssopifolium* (60%), *O. sanctum* (60%), *P. integerrima* (60%), *T. arjuma* (56%), *F. cretica* (48%), *T. indicum* (48%), *C. procera* milky exudates (44%), *C. cyminum* (44%), *P. roxburghii* (44%), *A. sativum* (40%) and *A. officinalis* (20%) are listed in descending order for neutralization of phospholipase A activity.

Seventeen per cent plant extracts failed to demonstrate their ethnobotanical claim as anti-venom. Merely 24% of

Table 1. Description of medicinal plants of Pakistan (their collected parts with voucher specimen deposited and voucher number) from different locations having ethnobotanical evidences as anti-venom

Medicinal plants	Botanical name	Areas of collection (part collected)	Voucher number	Phytochemicals reported	Refe-rences
Alliaceae	*Allium cepa* L.	Bhakkar (bulbs)	STW.42	Proteins (1.2 g), fibre (0.6 g), carbohydrates (11 g) and water content (86.8 g) per hundred grams of material	18
Anacardiaceae	*Pistacia integerrima*	Murree (galls)	STW.458	Galls contain 1.3% essential oil having A-pinene, camphene, d-limonene, cineole, A-terpineol, aromadendren and caprylic acid	19
Apiaceae	*Cuminum cyminum* L.	Sargodha (seeds)	STW.516	Cumin oil (sminaldehyde, 1,3-p-menthadien-7-al, 1,4-p-menthadien-7-al) and essential oil	19
Apocynaceae	*Nerium indicum* Mill	Haripur (leaves and roots)	STW.564	Neriodorin, karabin, nerioderin, odorin, neriodorin and karabin	11
Apocynaceae	*Rhazya stricta* Dcne	Lakki Marwat (leaves)	STW.565	Triterpenes (Mg quinate, β-sitosterol and urosoic acid), glycosides (3-7-rhamnoside, isorhamnetin-3-7-rhamnoside and roblnin), alkaloid (sewarine), flavonoids (rhazianosides A and B), among enzymes: NADPH dependent tetrahydroaistonine and strictosidine synthase	11
Asclepiadaceae	*Calotropis procera* (Wild.) R.Br	Haripur (milky latex)	STW.566(a)	Calotropin, calotropagenin, uscharin, calotoxin, calactin, sterol, resin and tannins	11
Asclepiadaceae	*Calotropis procera* (Wild.) R.Br	Haripur (flowers)	STW.566(b)	Calotropin, calotropagenin, uscharin, calotoxin, calactin, sterol, resin, oil and tannins	11
Bignoniaceae	*Stenolobium stans* (L.) D. Don	Haripur (roots)	STW.669	Phenolic acids, β-setosterol, triterpenoids-ursolic acid, α-amarine, oleanic acid, indole metabolizing enzymes, indole-oxygenase, zeaxanthin, luteinzeaxanthin and β-carotene	19
Boraginaceae	*Trichodesma indicum* (Linn) R.Br	Sind province (complete plant)	STW.604	Hexacosane, ethyl hexacosanoate and 21,24-hexacos-adienoic acid ethyl esters. Seed oil possesses oleic, linoleic, palmatic, stearic and linolenic acid	19
Caesalpinaceae	*Bauhinia variegate* L.	Haripur (roots)	STW.374	Gums, tannins, fatty oil, β-sitosterol, lupeol, kaempferol-3-glucoside and 5,7-dehydroxy, and 5,7-dimethoxy-flavanone-4-0-a-L-rhamnopyranosyl-β-D glucopyranosides	20, 21
Cruciferae	*Brassica nigra* (L. Koch)	Manshera (seeds)	STW.302	Glucoside, sinigrin and essential oil	19
Cruciferae	*Matthiloa incana* (L.) R.Br	Rawalpindi (seeds)	STW.322	Sulforaphene, oil rich in γ-linolenic acid, chlorphyll *a* and *b*, carotenoids, N, P, K, and Na ions	19
Cucurbitaceae	*Momordica charantia* L.	Abbottabad (fruits)	STW.706	Mycose, steroidal glucoside, vicine, momordicines I and II, momorcharaside A, B, cucubitane triterpenoids, cycloeucalenol, stigmasterol, spinasterol, taraxerol, momordicosides, lophenol, thiocyanogen, diosgenin, 24-methylencycloartenol, squalene, phenyl propanoids, stigmastadien-3-beta-ol, glucoside and carotenoids	19
Cucurbitaceae	*Citrullus colocynthis* Schard	Bahawalpur (fruits)	STW.702	Citrulluin, citrulluene, citrulluic acid, dihydric alcohol, citrullol, phydroxybenzyl, methylether, bitter oil, citbittol, elaterin, hentriacontane, saponins, various alkaloid, glycosides and tannins	19
Combretaceae	*Terminalia arjuma* Wight & Arn	Islamabad (barks)	STW.502	Arjunetin, arjunine, essential oil, calcium salts, aluminium and magnesium salts, colouring materials, reducing sugars, pyrocatachol (tannin) and a laactone, tomentosic acid, ellagic acid, arjunolic acid and β-sitosterol	19, 22
Fabaceae (Papilionaceae)	*Psoralea corylifolia* L.	Peshawar (seeds)	STW.418	Corylifolean, corylifolin, corylifolinin, raffinose, psoralidin, psoralen, isopsoralidin, isopsoralen, bavachin, isobavachin, bakuchiol, bavachinin, 7-Omethylbavachin, 4-O-methylbavachalcone, neobavaisoflavone, isobavachalcone, bavachromene, corylidin, traincontane, β-sitosterol-D-glucoside, bavachalcone, neobavachalcone, isobavachalcone, isoneobavachalcone, bakuchalcone, stigmasterol, psoralidin-2,3-oxide diacetate. Limonene, β-caryophylenoxide, α-elemene, linalool, 4-terpineol, angelicin, geranylacetate, bakuchiol and psoralene	19

(*Contd*)

Table 1. (*Contd*)

Medicinal plants	Botanical name	Areas of collection (part collected)	Voucher number	Phytochemicals reported	References
Gentianaceae	*Enicostemma hyssopifolium* (Willd.) Verdoorn	Jhelum (fresh plant)	STW.553	Betuline, swertioside, sylyswertin, swertiamarin, isoswertisin, enicoflavine, erythrocentaurine, apigenin, genkwanin, isovetexin, swertisin and saponarin	20
Labiatae (Lamiaceae)	*Leucas capitata* Desf	Rawalpindi (complete plant)	STW.615	Essential oil and alkaloids	21
Labiatae (Lamiaceae)	*Ocimum sanctum*	Islamabad (complete plant)	STW.626	Essential oil containing 3.2% carvacrol, 71.3% eugenol, 1.7% caryophyllene, 20.4% methyl eugenol, linalool, methyl chavicol cineole and eugenol methol ether	22
Liliaceae	*Allium sativum* L.	Bhakkar (bulbs)	STW.46	Allicin, thiosulfinates, anthocyanins, 2 mercapto-L-cysteins, polysaccharides, allinase, sativin I and II, quercetin, glycosides of kaempferol and scordinines A and B	23
Malvaceae	*Althaea officinalis* L.	Rawalpindi (roots)	STW.411	Asparagines, sucrose, phenolic acids, fatty oil, butyric acid, phytosterin, flavonoids, pectin (11%), starch (37%) and 11% mucilage	11
Mimosaceae	*Albizia lebbeck* (L.) Benth	Bahawalpur (seeds)	STW.381	Alkaloid, tannins, carbohydrate, flavanoids, proteins, echinocystic acid and amino acids	19
Pinaceae	*Cedrus deodara* G. Don	Nathia Gali (bark)	STW.25	Essential oil, cholesterin, gum, ascorbic acid, himachalol, allohimachalol, himadarol, cantdarol, cedrinoside dihydrodehydrodiconiferyl alcohol, cedrin, isocentdarol, dihydromyricetin, dewarene, dewarol, dewardiol and taxifolin	19
Pinaceae	*Pinus roxburghii* Sargent	Murree (oleoresins)	STW.26	β-carene, α-pinene, β-longifolene, β-pinene, longifolene, longicyclene and careen	19
Rubiaceae	*Rubia cordifolia*	Murree (stems)	STW.689	Purpurin and munjistin. Apart of it other chemical substances are pseudopurpurin and xanthopurpurin. Alizarin and munjistin with their glycosides	19
Rutaceae	*Citrus limon* (L.). Burm. F	Haripur (fruit)	STW.xx	Essential oil, d-x-pinene camphene, d-limonene, linalool, ichangin 4-β-glucopyranoside, nomilinic acid and 4-β-glucopyranoside	24
Sapindaceae	*Sapindus mukorossi* Gaertn	Rawalpindi market (fruits)	STW.463	Kaempferol, β-sitosterol and quercetin. Among saponins: sapindoside A, sapindoside B and saponin emarginatoside	25
Zingibesraceae	*Zingiber officinalis* Rosc	Lahore (rhyzome)	STW.66	Camphene, cineol, singiberine, shagaol, potassium oxalte, citral borneol, gingerol, β-phellandrene, α-curcumene, α-bergamotene, β-D-curcumene and gamma-bisabolene	26
Zygophyllaceae	*Fagonia cretica* L.	Lasbella (leaves and twigs)	STW.433	Saponin glycosides, saponin-I and saponin-II, various proteins from aqueous extract, docosyl docosanoate from *n*-hexane extract, ursolic acid, pinitol and nahagenin	13

the tested extracts were involved to reverse phospholipase A enzymatic action with less than 50% efficiency. However, a large percentage (48%) of plant extracts was found to neutralize anticoagulation phenomenon of phospholipases A with more than 50% in competency. Eleven per cent of plants were found to match their folklore claim as anti-venom.

Phospholipases are the most copious and tiny molecules among snake venom enzymes that cause havoc by halting normal physiological processes and pose intricate challenges to protein scientists. Venom from various snakes has been reported previously in the literature to show anticoagulant property by virtues of phospholipase A. These include *Bitis gabonica*, *Crotalus admanteus*, *Naja melanoleuca*, *Naja nigriccollis*, *Naja naja* and *Vipera berus orientale*. Phospholipase A has been sequenced for its amino acids to recognize intriguing mechanism of anticoagulation rendered by 54–77 residues. They may be strong or weak anticoagulants due to inhibition of extrinsic tenase or prothrombinase complex in prothrombin time (PT) prolongation or incapable of conversion of fibrinogen to fibrin in thrombin time (TT) tests[8]. Similarly, to assure the presence and action of phospholipase A as an anticoagulant, venom from *N. n. karachiensis* was tested using egg yolk mixture *in vitro* coagulation assay. Phospholipases were involved to delay coagulation of egg yolk mixture in contrast to venom-free sample (control). Additionally *in vitro* reports of prolongation (published previously by our group) about PT and TT stemmed to assume thrombin-like action(s) of these enzymes instead of having strong or weak anticoagulant effect[17].

Anticoagulant effect of phospholipase A is due to enzymatic and non-enzymatic action. Enzymatic action by which phospholipase A shows anticoagulant effect is enzyme–substrate binding mechanism. Phospholipase A

Table 2. Neutralization of anticoagulant effect induced by weak phospholipase A (PLA) enzymes present in *Naja naja karachiensis* venom using medicinal plants of Pakistan (PLA results are mentioned as mean ± standard deviation, $n = 3$)

Name of sample and concentration of each sample (5 µg/200 µl)	Coagulation time (s)	PLA enzyme activity (%)	PLA enzyme inhibition (%)
Naja naja karachiensis venom	125 ± 0.57	100	0
Control (saline)	100 ± 1.00	0	100
Standard anti-dote (antisera)	100 ± 0.57	0	100
Albizia lebbeck (L.) Benth	125 ± 1.52	100	0
Allium cepa L.	106 ± 0.57	24	76
Allium sativum L .	115 ± 1.52	60	40
Althaea officinalis Linn	120 ± 0.57	80	20
Bauhinia variegate L.	108 ± 1.52	24	76
Brassica nigra (L. Koch)	125 ± 1.52	100	0
Calotropis procera (Wild.) R.Br (exudates)	114 ± 0.00	56	44
Calotropis procera (Wild.) R.Br (flowers)	106 ± 1.00	24	76
Cedrus deodara G. Don	105 ± 1.52	20	80
Citrullus colocynthis	100 ± 0.57	0	100
Citrus limon (L.). Burm. f.	109 ± 1.00	36	64
Cuminum cyminum L.	114 ± 2.00	56	44
Enicostemma hyssopifolium (Willd.) Verdoorn	110 ± 1.00	40	60
Fogonia cretica L.	113 ± 0.57	52	48
Leucas capitata Desf.	105 ± 0.00	20	80
Matthiloa incana (L.) R.Br	125 ± 1.52	100	0
Momordica charantia L.	106 ± 1.00	24	76
Nerium indicum Mill	125 ± 0.57	100	0
Ocimum sanctum	110 ± 2.00	40	60
Pinus roxburghii Sargent	114 ± 1.52	56	44
Pistacia integerrima	110 ± 1.00	40	60
Psoralea corylifolia L.	102 ± 0.57	8	92
Rhazya stricta Dcne	125 ± 1.00	100	0
Rubia cordifolia	100 ± 1.00	0	100
Sapindus mukorossi Gaertn	106 ± 2.51	20	80
Stenolobium stans (L.) D. Don	100 ± 0.57	0	100
Terminalia arjuna Wight & Arn	111 ± 1.15	44	56
Trichodesma indicum (Linn) R.Br	113 ± 0.57	52	48
Zingiber officinalis Rosc	102 ± 1.00	8	92

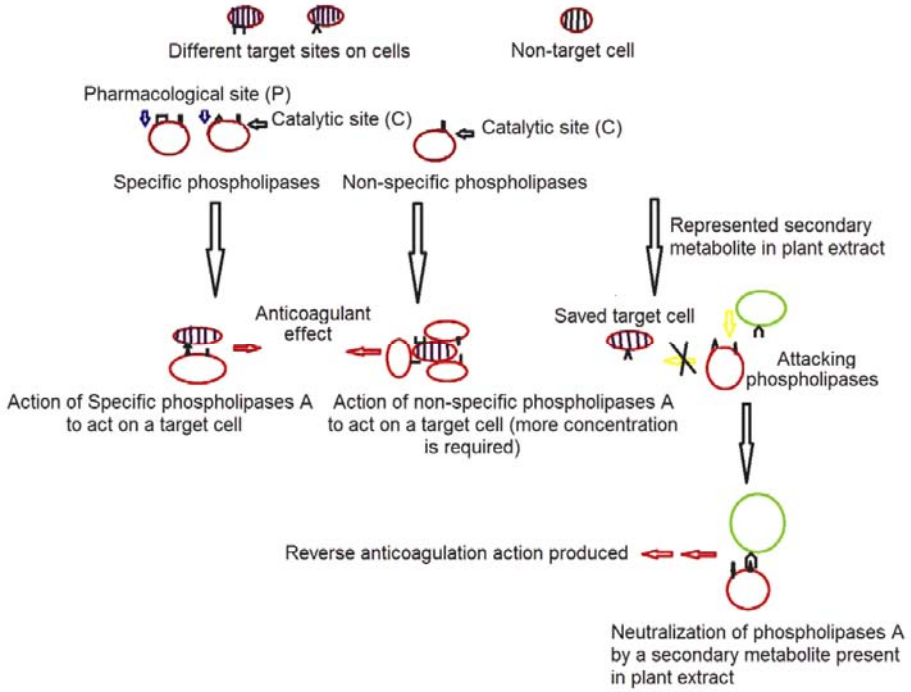

Figure 1. Proposed mechanism of action of phospholipase A and its neutralization by secondary metabolites of medicinal plants[3].

is abundant in pharmacological (p) and catalytic (c) sites on its surface (specific phospholipases) or solely catalytic sites (non-specific phospholipases). These sites easily recognize their complementary target sites (glycoproteins or phospholipids) on the target cells or tissues. Phospholipids will be hydrolysed into intact phospholipids or free fatty acids and lysophospholipids, thus clearly indicated anticoagulant behaviour. Sometimes phospholipases and target protein complexes are formed and act as agonist, antagonist or produce obstruction in binding of normal physiological ligand to its target protein; therefore demonstrating anticoagulant activity[3]. The overall mechanism of anticoagulation is shown in Figure 1.

Enzyme-inhibiting and protein-binding compounds have acquired therapeutic significance of natural inhibitors to cope with snake venom envenomation. Pakistani medicinal plants have primordial record to turn off such enzymes due to various secondary metabolites. Among them, flavonoids, phenols, quinonoids, terpenoids and xanthenes were documented previously to deactivate snake venom constituents[11]. Plant secondary metabolites react and sequester phospholipases and pose hindrance in binding of the latter to their target site(s) hence reverse anticoagulation action of phospholipase A. Thus the present study has demonstrated successfully the ethnobotanical claims (as anti-venom) about medicinal plants of Pakistan according to their efficiency (100% to 0%) to rationalize them logically in the traditional system of medicine. However, further studies are inevitable for the discovery and isolation of bioactive constituent(s) from these cited medicinal plants. Snake venom constituent (phospholipase A) which has bestowed thrombin-like action(s) to this venom should be studied in greater detail for its activities.

1. Zouari-Kessentini, R., Srairi-Abid, N., Bazaa, A., Ayeb, M. E., Luis, J. and Marrakchi, N., Antitumoral potential of Tunisian snake venoms secreted phospholipases A_2. *BioMed. Res. Int*., 2013, **19**, 1–9.
2. Dhananjaya, B. L., Nataraju, A., Rajesh, R., Raghavendra, G., Sharath, B. K., Vishwanath, B. S. and D'Souza, C. J. M., Anticoagulant effect of *Naja naja* venom 5′ nucleotidase: demonstration through the use of novel specific inhibitor, vanillic acid. *Toxicon*, 2006, **48**, 411–421.
3. Kini, R. M., Excited ahead: structure, function and mechanism of snake venom phospholipases A_2 enzymes. *Toxicon*, 2003, **42**, 827–840.
4. Waite, M., *Phospholipases in Biochemistry of Lipids, Lipoproteins and Membranes* (eds Vance, D. E. and Vance, J.), Elsevier, London, 1996, p. 211.
5. Dennis, E. A., Diversity of group types, regulation and function of phospholipase A_2. *J. Biol. Chem.*, 1994, **269**, 13057–13060.
6. Dennis, E. A., The growing phospholipase A_2 superfamily of signal transduction enzymes. *Trends Biochem. Sci*., 1997, **22**, 1–2.
7. Burke, J. E. and Dennis, E. A., Phospholipase A_2 structure/function mechanism and signaling. *J. Lipid Res*., 2009, **50**, 237–242.
8. Kini, R. M., Structure–function relationships and mechanism of anticoagulant phospholipase A_2 enzymes from snake venoms. *Toxicon*, 2005, **45**, 1147–1161.
9. Chethankumar, M., Turmerin, a protein from *Curcuma longa* L. prevents oxidative organ damage against *Naja naja* venom phospholipase A_2 in experimental animal. *J. Curr. Pharm. Res*., 2010, **3**, 29–34.
10. Wadood, A., Ali, S. A., Sattar, R., Lodhi, M. A. and Zaheer-ul-Haq, M., A novel pharmacophore model to identify leads for simultaneous inhibition of anti-coagulation and anti-inflammatory activities of snake venom phospholipase A_2. *Chem. Biol. Drug Des*., 2012, **79**, 431–441.
11. Asad, M. H. H. B. *et al.*, Enlisting the scientifically unnoticed medicinal plants of Pakistan as a source of novel therapeutic agents showing antivenom activity. *Afr. J. Pharm. Pharmacol*., 2011, **5**, 2292–2305.
12. Khan, T., Ahmad, M., Ahmad, W., Saqib, Q. N. and Choudhary, M. I., Preliminary evaluation of the antispasmodic and lipoxygenase inhibitory effects of some selected medicinal plants. *Pharm. Biol.*, 2009, **47**, 1137–1141.
13. Razi, M. T., Asad, M. H. H. B., Khan, T., Chaudhary, M. Z., Ansari, M. T., Arshad, M. A. and Najam-us-Saqib, Q., Antihaemorrhagic (Antivenom) potentials of *Fagonia cretica* against Pakistani Cobra venom (*Naja naja karachiensis*). *Nat. Prod. Res*., 2011, **25**, 1902–1907.
14. Asad, M. H. H. B. *et al.*, Coagulopathies in *Naja naja karachiensis* (black Pakistan cobra) bites and its effect on coagulation tests upon storage of platelet poor plasma. *Acta Pol. Pharm. Drug Res*., 2012, **69**, 1031–1034.
15. Chakrabarty, D., Datta, K., Gomes, A. and Bhattacharyya, D., Haemorrhagic protein of Russell's viper venom with fibrinolytic and esterolytic activities. *Toxicon*, 2000, **38**, 1475–1490.
16. Mendes, M. M. *et al.*, Anti-snake venom properties of *Schizolobium parahyba* (Caesalpinoideae) aqueous leaves extract. *Phytother. Res.*, 2008, **22**, 859–866.
17. Asad, M. H. H. B., Razi, M. T., Murtaza, G., Azhar, S., Khan, S. A., Najam-us-Saqib, Q. and Hussain, I., Antihaemorrhagic potential of *Citrullus colocynthis* Schrad (Cucurbitaceae) against *Naja naja karachiensis* (black Pakistan cobra) venom. *J. Med. Plants Res*., 2012, **6**, 3455–3458.
18. Makhija, I. K. and Khamar, D., Anti-snake venom properties of medicinal plants. *Der. Pharm. Lett*., 2010, 2, 399–411.
19. Baquar, S. R., *Medicinal and Poisonous Plants of Pakistan*, Printas, Karachi, Pakistan, 1989, 1st edn, p. 21.
20. Daniel, M., *Medicinal Plants: Chemistry and Properties. Part A: Secondary Metabolites* (*Alkaloids*), Science Publishers, Enfield, NH, USA, 2006, p. 37.
21. Shinwari, M. I. and Shah, M., *Medicinal Plants of Margalla Hills National Park Islamabad*, D. G. Administration, Higher Education Commission, Islamabad, Pakistan, 2007, 1st edn, pp. 35–94.
22. Prajapati, N. D., Purohit, S. S., Sharma, A. K. and Kumar, T., *A Handbook of Medicinal Plants: A Complete Source Book*, Agrobios, Jodhpur, India, 2010, p. 367.
23. Rita, P., Animesh, D. K., Aninda, M., Benoy, G. K. and Sandip, H., Snake bite, snake venom, anti-venom and herbal antidote – a review. *Int. J. Res. Ayurveda Pharm*., 2011, **2**, 1060–1067.
24. Ugulu, I., Traditional ethnobotanical knowledge about medicinal plants used for external therapies in Alasehir, Turkey. *Int. J. Med. Aromat. Plants*, 2011, **1**, 101–106.
25. Parganiha, R., Verma, S., Wani, V., Deshmukh, V. S. and Sawarkar, H. A., *In vitro* antiasthamatic activity of fruits extracts of *Sapindus mukorossi* and *Piper nigrum*. *Int. J. Herbal Drug Res*., 2012, **1**, 12–16.
26. Duke, J. A. and Ayensu, E. S., *Medicinal Plants of China. Medicinal Plants of the World. Vol. 1*, Reference Publications Inc., Algonac, Michigan, USA, 1985, p. 362.

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