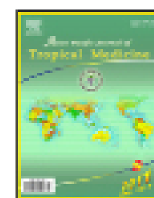
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm



Document heading doi:

Antihaemolytic and snake venom neutralizing effect of some Indian medicinal plants

Chidambaram Kumarapppan[1*], Albert Jaswanth[2], Karpagam Kumarasunderi[2]

[1]Department of Life Sciences, International Medical University, Kuala Lumpur, Malaysia
[2]Department of Pharmacology, Periyar College of Pharmaceutical Sciences, Tamilnadu, India

ARTICLE INFO

Article history:
Received 12 May 2011
Received in revised form 11 July 2011
Accepted 25 July 2011
Available online 20 September 2011

Keywords:
Antivenom activity
Naja nigricollis
hemolysis
neutralization
Indian medicinal plants

ABSTRACT

Objective: To validate traditional claims of usefulness of the Indian plants in management of poisonous snakebite and evaluate the antivenom properties displayed by the alcoholic extracts of *Andrographis paniculata* (*A. paniculata*), *Crateva magna* (*C. magna*), *Gloriosa superba* (*G. superba*) and *Hydrocotyle javanica* (*H. javanica*). **Methods:** These plants were collected, identified and the extracts were prepared by using conventional Soxhlet ethanol extraction technique. The venom neutralization activity was accessed in mice (20-25g) and number of mortalities was observed against clinically important snake (Naja nigricollis) venom. Present study also deals with in vitro membrane stabilizing activity of these plants against hyposaline induced human red blood corpuscles (HRBC). **Results:** Extracts of *H. javanica* and *G. superba* gave 80 % and 90 % protection to mice treated with minimum lethal dose of venom (LD_{99}). These two plants showed significant neutralization effect against the venoms of Naja nigricollis venom. *H. javanica* and *G. superba* (25-100 mg/mL) produced significant changes of membrane stabilization of human red blood cells (HRBC) exposed to hyposaline-induced haemolysis. **Conclusions:** We conclude that probably due to presence of various phytochemicals plays an important role in the anti-venom potential of these Indian medicinal plants against Naja nigricollis venom. The above observations confirmed that *A. paniculata*, *C. magna*, *G. superba* and *H. javanica* plant extracts possess potent snake venom neutralizing capacity and could potentially be used as an adjuvants for antivenin therapy in case of snakebite envenomation, especially against the local effects of cobra venoms.

1. Introduction

Snake envenoming is a major public health issue in the rural tropics with large numbers of envenoming and deaths especially in India. Poisonous snakebites in southern India are usually due to several types of snakes. The snakes particularly responsible for serious medical emergencies are the *Naja* species [mainly by *Naja nigricollis* Broadley (Elapidae) (*N. nigricollis*) and *Naja katiensis* Broadley & Hallowell (Elapidae)], and less commonly by Naja melanoleuca Rodel. Envenomation by the *Naja* spp. can cause severe local symptoms that include swelling, blistering, and necrosis with general symptoms of systemic envenomation; neurotoxicity is common. Snake venom antiserum development and standardization is expensive and requires ideal storage conditions; storage facilities for antiserum may be lacking in the usually remote snake endemic areas of India[1]. They can also induce adverse reactions ranging from mild symptoms to serious ones and they do not neutralize the local tissue damage[2].

Tribes of southern India have several claims of effective use of plants in treatment of poisonous snakebites including bites due to *N. nigricollis*, the most common of the spitting cobras[3,4]. The plant treatments are particularly popular, especially for prophylaxis of snakebites during the rainy season when the incidence of snakebites is usually high. The use of plants as alternatives for treatment of poisonous bites is important in remote areas where there is no accessibility to hospitals and storage facilities for anti-venom. Over the years many attempts have been made for the development of snake venom antagonists especially from plant sources. The use of plants against the effects of snakes bite has been long recognized; more scientific

*Corresponding author: Chidambaram Kumarapppan, Department of Life Sciences, School of Pharmacy & Health Sciences, International Medical University, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia, HP: 016-2242696.
E-mail: Kumarappan_chidambaram@imu.edu.my

attention has been given since last 20 years[5]. Extracts from plants have been used among traditional healers, especially in tropical areas where there are plentiful sources, as therapy for snakebite for a long time. Several medicinal plants, which appear in old drug recipes or which have been passed on by oral tradition, are believed to be snakebite antidotes[6].

In modern science, there have been many attempts to study these plants to clarify their effectiveness. India has a rich tradition of the usage of medicinal plants. Many Indian medicinal plants are recommended for the treatment of snakebite[7-9]. *Hemidesmus indicus* root extracts effectively neutralized Viper venom induced lethal, haemorrhagic, coagulant, anticoagulant and inflammatory activity. Lupeol acetate isolated from the root extract of Indian sarsaparilla *Hemidesmus indicus* R.Br. could significantly neutralize lethality, haemorrhage, defibrinogenation, edema, PLA2 activity induced by *Daboia russellii* venom. It also neutralized *Naja kaouthia* venom induced lethality, cardiotoxicity, neurotoxicity and respiratory changes in experimental animals[10,11]. The anti-snake venom plants contain more than one compound (secondary metabolites) that are responsible for venom neutralization. Thus medicinal plants with antivenom activity could be considered as an effective alternative to mammalian antibody production for the treatment of snakebite envenomation. This investigation reports antihaemolytic activity and venom neutralizing effect of some Indian plant extracts Including *Andrographis paniculata* (*A. paniculata*), *Crateva magna* (*C. magna*), *Gloriosa superba* (*G. superba*) and *Hydrocotyle javanica* (*H. javanica*). against the venom of *N. nigricollis*, one of the snake responsible for quite a number of fatal bites in southern India.

2. Materials and methods

2.1. Plant materials

A. paniculata (Acanthaceae), *C. magna* (Capparidaceae), *G. superba* (Liliaceae) and *H. javanica* (Apiaceae) were collected in the month of June 2008. The twigs of the plant along with flowers were submitted and Chief botanist at Department of Botany, St Joseph's college, Trichirappalli, authenticated it. Voucher specimens have been deposited in the herbarium of the Pharmacology Department, Periyar College of Pharmaceutical Sciences, Thiruchirappalli, India.

2.2. Preparation of plant extracts

Shade dried and powdered materials of *A. paniculata* (Leaves), *C. magna* (Stem Bark), *G. superba* (Tubers) and *H. javanica* (Leaves) were passed in sieve 22. The powered material was extracted using 95% ethanol in Soxhlet apparatus. The alcoholic extract obtained was filtered and the process was repeated for four days. The resulting filtrates were pooled for further processing. This pooled ethanolic extract was concentrated on rotavapour (Buchi R-114) and subjected to freeze drying in a Lypholizer (Heto Fd 3 drywinner). The dried extract was weighed to calculate the yield. This extract (yield 6.67%) and the dried plant extracts were freshly dissolved or suspended in normal saline prior to administration. The preliminary phytochemical analysis of these extracts was assayed using the standard methods and techniques[12].

2.3. Source of venoms and ethics

The venom of *Naja nigricollis* was purchased from Kings institute, Chennai, India. The experiments were approved by the institutional ethical animal committee (IEAC) of Periyar College of Pharmaceutical Sciences, Thiruchirappalli and are in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment & Forests. (Animal Welfare Division), New Delhi, India.

2.4. Animals

Swiss albino mice (20-25 g) of either sex, maintained in the Animal Experimental Laboratory of Periyar College of Pharmaceutical Sciences, at room temperature of (25 ±2) ℃, relative humidity of (75±5) % and 12 h dark-light cycle. They were fed with standard animal pellets (Hindustan Animal Feeds, Bangalore, India) and allowed free access to water (*ad libitum*). The project was approved by Institute Animal Ethical Committee. Each experimental group consisted of six animals housed in separate cages. The animals had access to standard laboratory feed (M/s. Hindustan Lever Ltd.)

2.5. Anti-venom activity and venom neutralization

2.5.1. Lethal toxicity assay

The median lethal dose (LD_{99}) of *N. nigricollis* venom was determined according to the previously developed method[1]. Various doses of venom in 0.2 mL of physiological saline was injected into the tail vein of mice, using groups of 10 mice for each venom dose. The LD_{99} was calculated with the confidence limit at 50% probability by the analysis of deaths occurring within 24 h of venom injection. The antilethal potentials of *A. paniculata*, *C. magna*, *G. superba* and *H. javanica* plant extracts were determined against LD_{99} of *N. nigricollis* venom. Various amount of plant extracts (μL) were mixed with LD_{99} of venom sample and incubated at 37 ℃ for 30 minutes and then injected intravenously into mice. Five (n=10) mice were used at each antivenom dose. Control mice received same amount of venom without antivenom (plant extracts). The neurotoxicity and the number of deaths were calculated within 24 h of injection of the venom/antivenom mixture.

2.5.2. Detoxification of venom by extracts

Five groups of mice (n=10) were used in this study. The first group received 0.2 ml of the MLD (LD_{99} 900 μg/kg) of *N. nigricollis* venom only. Groups 2-5 (serving as treatment groups) were given an equivalent of the MLD of the venom containing 50 mg of the extract. The venom and the extract were incubated at 37 ℃ for 10 min and 0.2 mL of the incubated mixture was injected (ip) into each animal in the treatment groups. The number of deaths was recorded within 24 h.

2.6. Hyposaline induced haemolysis

The hyposaline induced haemolysis was evaluated *in vitro* by the previously described method[13]. Blood was collected from healthy adult human volunteers in sterile Alsever's solution and used within 5 hrs of its blood collection. The preparation of cell suspension was carried out as previously described method[14]. Hyposaline (0.36 %, 2 mL), phosphate buffer (0.15 m, pH 7.4 1 mL) and HRBC (1%, 0.5 mL) were taken in various test tubes. To the above tubes different concentrations of the drugs were added. The drugs were prepared using isolsaline solution. The tube containing isosaline (0.85%) served as control. All the tubes were incubated at 37 ℃ for 30 min and centrifuged. The color of the supernatant (due to hemoglobin release) was measured at 560 nm. The control was taken as 100 percent lyses and the percentage of prevention of haemolysis of the drug was calculated using the relation

$$\text{Percentage prevention of haemolysis}=\frac{\text{Absorbance of treated sample}}{\text{Absorbance of the control}}\times 100$$

2.7. Statistical analysis

The significance of difference among the various treated groups and control group were analyzed by means of one-way ANNOVA followed by Dunnett's multiple comparison test using Graphat Instat Software (SanDiego, CA, USA). The results were expressed as the mean ±SEM of the number of experiments done, with $P<0.05$ indicating significance.

3. Results

The preliminary phytochemical analysis of these plant extracts revealed the presence of steroids, flavonoids, alkaloids and tannins. The anti-haemolytic property of the ethanolic extracts of *A. paniculata, C. magna, G. superba* and *H. javanica* were assessed through inhibition of in vitro HRBC lysis and the results were shown in Figure 1 and 2. The invitro assay of *A. paniculata, C. magna, G. superba* and *H. javanica* were inhibited hypotonicity induced HRBC membrane lysis by 50.99 %, 60.89 %, 66.60 % and 74.21 %, respectively at a concentration of 10 μg/mL of the extracts. Among the four extracts alcoholic extract of *H. javanica* showed the maximum inhibitory activity against hypotonicity induced HRBC haemolysis. Blood hemolysis by venoms is mostly due to the phospholipase enzymes[15]. The minimal lethal dose (MLD) of *N. nigricollis* venom was 900 μg/kg (estimated by probit analysis). Mice treated with MLD of venom showed excitement followed by respiratory depression, paralysis (especially of the hind limb), coma and death usually accompanied these symptoms. These are classical symptoms of neurotoxicity. Table 1 shows the effect of 50 mg/ kg extracts against the minimum lethal dose of venom. Survival % (protection) calculated over 24 h showed extracts of *H. javanica* and to *G. superba* have offered the highest protection (80% and 90%, respectively), as compared with *A. paniculata* and *C. magna* (40% and 60%, respectively). The extracts of *G. superba* and *H. javanica* significantly alleviated the toxic signs of the venom and offered significant ($P<0.01$) protection against *N. nigricollis*. Plant constituents are known to neutralize venom component *in vivo*[16].

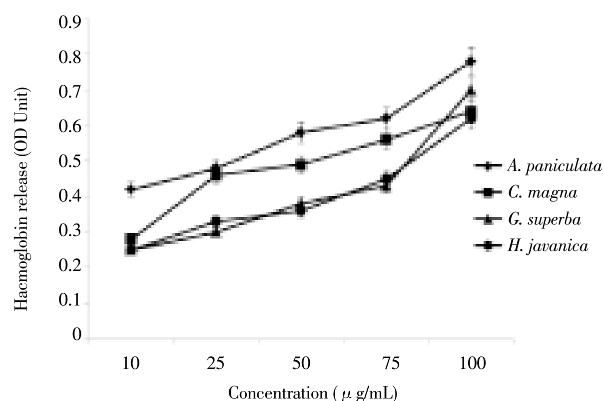


Figure 1. Effect of ethanolic extracts of *A. paniculata, C. magna, H. javanica, G. superba* on hyposaline induced hemoglobin release in HRBC.
Values are expressed as mean ± SEM of triplicate experiments.

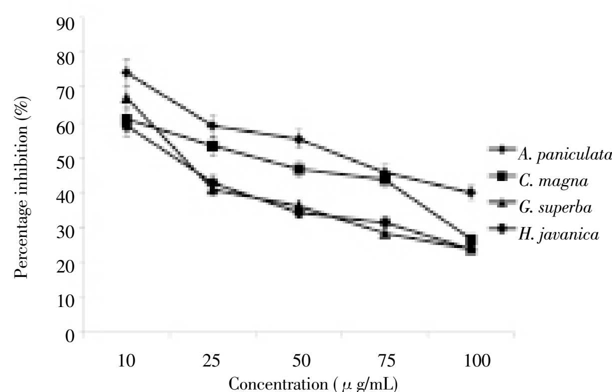


Figure 2. Effect of ethanolic extracts of *A. paniculata, C. magna, H. javanica, G. superba* on hyposaline induced haemolysis in HRBC.
Values are expressed as mean ± SEM of triplicate experiments.

Table 1
Effect of administration (ip) incubated mixture of *N. nigricollis* venom and ethanolic extracts (50 mg/kg) in Swiss albino mice.

Treatment Groups	Number of deaths/number of mice used	Survival %(within 24 hr)	Signs of neurotoxicity
LD_{99}	10/10	……	+++
LD_{99} + C. magna	6/10	40^{*}	++
LD_{99} + H. javanica	2/10	80^{**}	+
LD_{99} + A. paniculata	4/10	60^{*}	++
LD_{99} + G. superba	1/10	90^{**}	+

n=10, Significance ** $P<0.01$, * $P<0.05$ when compared with venom treated animals. + indicates difficulty in breathing; ++ indicates difficulty in breathing and impaired movement; +++ indicates severe respiratory impairment and coma.

4. Discussions

Snakebites constitute a health problem in many tropical and subtropical countries, with an estimated 2.5 million people envenomed each year worldwide[17,18]. Envenoming by *N. nigricollis* is responsible for several clinical complications of severe systemic and local pathology. On the other hand envenoming by *N. nigricollis* induced clinical complications different from other snake venoms. These include local necrosis, haemorrhage, complement depletion and respiratory arrest or paralysis[19, 20]. Although an intravenous administration of antivenom, prepared from IgG of venom-immunised horses or sheep, is an effective treatment for systemic envenoming against *N. nigricollis*, the clinical consensus is that antivenom is of limited effectiveness against the effects of local envenoming that develop rapidly after a bite. Research to develop a treatment for local envenoming is therefore a clinical priority and has focused on the application of natural or synthetic inhibitors of snake venom potent molecules[21]. Plants used as remedy for snakebite abound in literature[22].

Consequently, the search for novel natural or synthetic molecules capable of inhibiting the local damage caused by envenoming is a promising field of research. Plant extracts provide an alternative for the treatment of snakebites, principally because of the large diversity of pharmacologically compounds that they contain. However, in most cases, the use of these plants requires scientific validation to confirm their supposed antivenom activities. The ability of plant extracts to neutralize venom has been attributed to the presence of plant "secondary metabolites" capable of binding venom proteins and inhibiting enzyme activity[23-26]. Such a mechanism may well be involved in the action of many phytochemicals in these plant extracts and could explain the 'protective' effects of plant extracts when preincubated with venom before testing in biological assays[27].

Flavonoids, quinonoids, xanthenes, polyphenols and terpenoids, which are plant secondary metabolites, possess protein binding and enzyme inhibiting properties and also inhibit snake venom PLA2[28]. Although component(s) of these plant extracts responsible for the antivenom activity observed in the present study has not yet been identified and isolated to verify its anisnake venom potential. The neutralization ability of snake antivenoms is still assessed by the traditional in vivo lethality assay, comparable to those used for bacterial antitoxins, usually performed in mice[29]. This prospect was confirmed partially by "protective" effects of these plant extracts when they are preincubated with *N. nigricollis* venom before administration to the biological assay. Interactions between animal venoms and plant constituents have been described, but an adequate understanding of the mechanisms involved requires study of the isolated compounds.

Snake bite also causes haemolysis which is one of the contributing factors of snake poison. The enzyme PLA2 acts on the human red blood cells (HRBC) membrane associated phospholipids liberating lysolecithin causes haemolysis. The in vitro oxidative hemolysis of HRBC was used as a model to study the free radical induced damage of biological membranes and the inhibitory effect of natural antioxidants. Exposure of HRBC to injurious substances such as hypotonic medium, methyl salicylate or phenyl hydrazine results in the lysis of membrane accompanied by haemolysis and oxidation of haemoglobin[30-32]. The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. Injury to RBC membranewill render the cell more susceptible to secondary damage through free radical-induced lipid peroxidation. This action is consistent with the observation that breakdown of biomolecules leads to the formation of free radicals which in turn enhance cellular damage[33]. It has also been reported that, there is production of free radicals, such as lipid peroxide and superoxide in various conditions, such as stress induced haemolysis, due to cell membrane destabilization[34].

This study, also demonstrated capability of the extract, to stabilize red blood cell membrane, which is an indication of the extracts ability to prevent rupture, or haemolysis in hypotonic-stress induced condition. The exact mechanism of action, responsible for the membrane stabilizing activity of the plant extract, could not be established in this study. Presence of polyphenolic flavonoids are the possible candidates that might explain the antioxidant activity of these extracts. In fact, when intact human RBCs were preincubated with ethanolic extracts, a strong protective effect against hypotonic saline generated hemolysis was observed. However, further experimental studies are needed to document whether the extract is capable of protection against other oxidants in vitro as well as invivo.

Traditional herbal medicine is readily available in rural areas for the treatment of snakebite. Plants are used either single or in combination, as antidotes for snake envenomation by rural populations in India and in many parts of the world. In India, there are about 54 million indigenous people of different ethnic groups inhabiting various terrains. These indigenous groups possess their own distinct culture, religious rites, food habit and a rich knowledge of traditional medicine. Even today, indigenous and certain local communities practice herbal medicine to cure a variety of diseases, with plants particularly used as folk medicine to treat snakebites. In conclusion, this study has confirmed the ethnomedical use of *A. paniculata, C. magna, H. javanica, G. superba* for the treatment of snakebite victims in rural peoples in the Indian state of Tamilnadu. So plant remedies may be beneficial for the treatment of snakebite and may find alternative to antivenom serum.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors thank our correspondent, Periyar College of Pharmaceutical Sciences, Periyar Maniammai University, Thiruchirappalli, Tamilnadu for his constant support and providing facilities to carry out this work successfully. King Institute of Preventive Medicine, Guindy, Chennai, is also thanked for the *Naja nigricollis* venom support.

References

[1] Soares AM, Ticlia FK, Marcussi S, Lourenço MV, Januário AH, Sampaio SV, et al. Medicinal Plants with Inhibitory Properties Against Snake Venoms. *Curr Med Chem* 2005; **12**: 2625-2641.

[2] Januário AH, Santos SL, Marcussi S, Mazzi MV, Pietro RCLR, Sato DN, et al. Neo-clerodane diterpenoid, a new metalloprotease snake venom inhibitor from *Baccharis trimera* (Asteraceae): Anti-proteolytic and anti-hemorrhagic properties. *Chem Biol Interact* 2004: **150**: 243-251.

[3] Soares AM, Januário AH, Lourenço MV, Pereira AM, Pereira PS. Neutralizing effects of Brazilian plants against snake venoms. *Drugs of the Future* 2004; **29**: 1105-1117.

[4] Haruna AK, Choudhury MK. *In vivo* antisnake venom activity of the furanoid diterpene from *Aristolochia albida* Duch. *Ind J Pharm Sci* 1995; **27**: 222-224

[5] Santosh RF, Shivaji PG. Preliminary screening of herbal plant extracts for anti-venom activity against common sea snake (Enhydrina schistosa) poisoning. *Phcog Mag* 2004;**16**: 56-60

[6] Alam MI, Gomes A. Snake venom neutralization by Indian medicinal plants (Vitex negundo and Emblica officinalis) root extracts. *J Ethnopharmacol* 2003; **86**: 75-80.

[7] Meenatchisundaram S, Parameswari G, Subbraj T, Michael A. Anti-venom activity of medicinal plants-a mini review. *Ethnobot Leaflets* 2008;**12**: 1218-1220.

[8] Mahanta M, Mukherjee AK. Neutralisation of lethality, myotoxicity and toxic enzymes of *Naja kaouthia* venom by *Mimosa pudica* root extracts. *J Ethnopharmacol* 2001;**75**: 55-60.

[9] Pithayanukul P, Laovachirasuwan S, Bavovada R, Pakmanee N, Suttisri R. Anti-venom potential of butanolic extract of *Eclipta prostrata* against Malayan pit viper venom. *J Ethnopharmacol* 2004; **90**: 347-352

[10] Chatterjee I, Chakravarty AK, Gomes A. *Daboia russellii* and *Naja kaouthia* venom neutralization by lupeol acetate isolated from the root extract of Indian sarsaparilla Hemidesmus indicus R.Br. *J Ethnopharmacol* 2006;**106**: 38-43.

[11] De Paula RC, Sanchez EF, Costa TR, Martins CHG, Pereira PS, Lourenço MV, et al. Antiophidian properties of plant extracts against Lachesis muta venom. *J Venom Anim Toxins Incl Trop Dis* 2010; **16**:311-323,

[12] Khandelwal KR. *Practical pharmacognosy*. Pune: Nirali Prakashan Publisher; 2006, p.149.

[13] Maiorano VA, Marcussi S, Daher MA, Oliveira CZ, Couto LB, Gomes OA, et al. Antiophidian properties of the aqueous extract of Mikania glomerata. *J Ethnopharmacol* 2005; **102**: 364-370.

[14] Oguiura N, Boni-Mitake M, Affonso R, Zhang G. *In vitro* antibacterial and hemolytic activities of crotamine, a small basic myotoxin from rattle snake *Crotalus durissus*. *J Antibiot* 2011;**64**: 1-5

[15] Liu S, Dong W, Kong T. Preparation and characterization of immunoglobulin yolk against the venom of *Naja naja* atra. *Ind J Exp Biol*. 2010; **48**:778-785

[16] Daduang S, Sattayasai N, Sattayasai J, Tophrom P, Thammathaworn A, Chaveerach A, et al. Screening of plants containing *Naja naja* siamensis cobra venom inhibitory activity using modified ELISA technique. *Anal Biochem* 2005; **341**:316-325.

[17] Doley R, Mukherjee AK. Purification and characterization of an anticoagulant phospholipase A(2) from Indian monocled cobra (*Naja kaouthia*) venom. *Toxicon* 2003; **41**: 81-91.

[18] Mukherjee AK, Maity CR. Biochemical composition, lethality and pathophysiology of venom from two cobras-- *Naja naja* and N. kaouthia. *Comp Biochem Physiol B Biochem Mol Biol* 2002; **131**:125-132.

[19] Da silva JO, Coppede JS, Fernandes VC, Sant'ana CD, Ticli FK, Mazzi MV, et al. Antihemorrhagic, antinucleolytic and other antiophidian properties of the aqueous extract from *Pentaclethra macroloba*. *J Ethnopharmacol* 2005;**100**: 145-152.

[20] Mohapatra B, Warrell DA, Suraweera W, Bhatia P, Dhingra N, Jotkar RM, et al. Snakebite mortality in India: A nationally representative mortality survey. *PLoS Negl Trop Dis* 2011; **12**;e1018.

[21] Perales J, Neves-Ferreira AG, Valente RH, Domont GB. Natural inhibitors of snake venom hemorrhagic metalloproteinases. *Toxicon* 2005; **45**:1013-1020.

[22] Amui SF, Puga RD, Soares AM, Giuliatti G. Plant-antivenom: Database of anti-venom medicinal plants. *Electr J Biotech* 2011;**14**: 1-9

[23] Rajani M, Shrivastava N, Ravishankara MN. A rapid method for isolation of andrographolide from andrographis paniculata nees (kalmegh). *Pharm Biol* 2000; **38**:204-209.

[24] Gagandeep Meera, Kalidhar SB. Chemical constituents of *Crataeva nurvala* (Buch-ham) leaves. *Ind J Pharml Sci* 2006; **68**: 804-806

[25] Singh B, Chandan BK, Sharma N, Bhardwaj V, Satti NK, Gupta VN, Gupta BD, et al. Isolation, structure elucidation and in vivo hepatoprotective potential of trans-tetracos-15-enoic acid from Indigofera tinctoria Linn. *Phytother Res* 2006; **20**:831-839.

[26] Joshi CS, Priya ES, Mathela CS. Isolation and anti-inflammatory activity of colchicinoids from *Gloriosa superba* seeds. *Pharm Biol* 2010; **48:**206-209.

[27] Pithayanukul P, Ruenraroengsak P, Bavovada R, Pakmanee N, Suttisri R, Saen-oon S. Inhibition of *Naja kaouthia* venom activities by plant polyphenols. *J Ethnopharmacol* 2005; **97**:527-533

[28] Asuzu AL, Harvey A. The antisnake venom activities of Parkia biglobosa (Mimosaceae) stem bark extract. *Toxicon* 2003; **43**:763-768

[29] Petras D, Sanz L, Segura A, Herrera M, Villalta M, Solano D, et al. Snake venomics of African spitting cobras: toxin composition and assessment of congeneric cross-reactivity of the pan-African EchiTAb-Plus-ICP antivenom by antivenomics and neutralization approaches. *J Proteome Res* 2011; **10**:1266-1280

[30] TK Mohamed Saleem, Azeem AK, Dilip C, Sankar C, Prasanth NV. Duraisami R. Anti-inflammatory activity of the leaf extacts of *Gendarussa vulgaris* Nees. *Asian Pac J Trop Biomed* 2011; **1**:147-149.

[31] Osonuga IO, Osonuga OA, Onadeko AA, Osonuga A, Osonuga AA. Hematological profile of pregnant women in southwest of Nigeria. *Asian Pac J Trop Dis* 2011; **1**(3): 232-234.

[32] Viswanathan S, Muthu V, Iqbal N. Upper lobe pulmonary edema and dry gangrene in scorpion sting. *Asian Pac J Trop Dis* 2011; **1**(2): 166-168.

[33] Arzanlou M, Bohlooli S, Jannati E, Mirzanejad-Asl H. Allicin from garlic neutralizes the hemolytic activity of intra- and extra-cellular pneumolysin O *in vitro*. *Toxicon* 2011; **57**: 540-545

[34] Agarwal BA, Rangari VD. Anti-inflammatory and anti-arthriticactivities of Lupeol and 19OH lupeol isolated from Strobilanthus callosus and Strobilanthusviocephala roots. *Indian J Pharmacol* 2003; **35**:384-387.