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Original Research

Anti-snake Venom Activities of Ethanol and Aqueous Extract of *Cassia hirsute* against Indian Cobra (*Naja naja*) Venom Induced Toxicity

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Abstract

Snake bite leads to medical emergencies and sometimes death. It is clinically managed by administration of monovalent/polyvalent antisera and it exhibit early or late adverse reactions and sometimes these adverse effects lead to fatalities. *Cassia hirsute* has been used against snake bite by the traditional healers; however, there is paucity of scientific data in support of this claim. To examine the anti-snake venom activities of ethanolic and aqueous extracts of *Cassia hirsute* against Indian cobra (*Naja naja*) venom induced toxicity. Anti-snake venom activities of ethanolic and aqueous extracts of *Cassia hirsute* against Indian cobra (*Naja naja*) venom induced toxicity was studied in mice, rats and sheep by using various models as follows: Phospholipase A_2 induced hemolysis on sheep RBC, *Naja naja* induced anticoagulation[Coagulation activity], *Naja naja* induced bleeding, *In- vivo* Neutralization activity against lethality induced by *Naja naja* venom (2mg/kg) in rats, *Naja naja* venom induced odema, liver function of venomized rats. The Minimum lethal dose (MLD) was found as 2mg/kg and both ACH and ECH shown significant neutralization of *Naja naja* venom induced lethality. Both extracts inhibited phospholipase A_2 dependent hemolysis of sheep RBC's and showed a high significant reduction (P<0.001) in the anti-coagulant activity of venom with 80.51% inhibition of anti-coagulation of ECH at 350 µg and 79.84% of ACH at 300 µg. ECH and ACH 300, 600 and 900 mg/kg shows significant (P<0.001) decrease in bleeding time also. These findings suggest a potential antivenom role of *Cassia hirsute* against *Naja naja* venom.

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INTRODUCTION

Snake bite is an occupational hazard in tropical and sub-tropical countries like India (Ahmed *et al*., 2008). It is estimated that 2.5 million people get envenomed each year on a global basis (Chippaux, 1998 and Sharma *et al*., 2008) and approximately 35,000- 50,000 deaths are reported in India per annum (Warrell, 1999 and Dhananjaya, 2011). An accurate measure of snake bite envenoming remains elusive as victims approach traditional healers for aid and treatment. Snake envenomation leads to many pathophysiological conditions like inflammation, hemorrhage, necrosis, nephrotoxicity, cardio toxicity and finally death (Theakston and Reid, 1983). The most common poisonous snakes found in India are Spectacled cobra (*Naja naja*), Krait (*Bungarus caeruleus*), Russell's viper (*Daboia russelli*) and Saw-scaled viper (*Echiscarinatus*). The cobra and krait belong to Elapidae family and their venom is considered to have predominantly neurotoxic effects, they affect the victim's central nervous system and cause heart failure. Their venom possesses several proteins, including cardiotoxins, neurotoxins and phospholipase A_2, that are responsible for their toxicity. Russell's viper and saw-scaled viper belong to Viperidae and are histotoxic (Bawaskar, 2001) hemorrhagic, therefore they provoke hemorrhagic manifestations that include epistaxis and cardiac manifestations such as myocarditis and cardiac failure (Meenatchisundaram and Michael, 2009). *Naja naja* (Indian Cobra) is commonly found in most of the Indian states and its bite leads to several deaths per year. Antivenom use against snake bites is lacking in the rural areas of Asian countries due to its cost and special storage conditions that are required for keeping a stock of antivenoms. Antiserum; being the only therapeutic agent, its development from animal source is time consuming and expensive. Availability of monovalent/polyvalent antiserum in rural areas is very less due to its high cost and special storage requirements. Additionally there are few hospital centers in rural areas that can manage snakebite cases. To overcome these drawbacks, there is a need to search, develop new affordable and safe antidote against snakebites.

The World Health Organization estimates that 80% of the world's population depends on traditional medicine for their primary health care needs. As long as man can remember, plants/plant materials have been used

worldwide in traditional medicine for the treatment of different diseases. It is estimated that even today approximately seventy percent of the world population rely on medicinal plants as their primary source of medicines (McChesney, 1995). Over the years, many attempts have been made for the development of snake venom antagonists especially from plant sources in spite of the existence of antiserum. Many Indian medicinal plants/plant materials are recommended for the treatment of snakebite (Chopra, Nayar and Chopra, 1956 and Nazimudeen, Ramaswamy and Kameswaran, 1978).

Cassia hirsute Linn. Plant (Holm *et al*., 1979 and Irwin and Barneby, 1982) belongs to the family *Caesalpiniaceae* and is commonly called as stinking cassia and hairy senna. It is a native of tropical America and now distributed in Malaysia, Indo-China, Thailand, Asian and African tropics, Laos, Java, Brazil, California, New Mexico and India. *Chakma* communities of Bangladesh use the leaves against snakebite (Roy, 2008). Leaf and root extract is used by the tribal people of Chittagong hill tracts of Bangladesh. Extracts are also used to prepare juice and paste. Juice is taken orally and paste is applied locally (Biswas *et al*., 2010). Also traditionally it is widely used for stomach troubles, dysentery, abscesses, rheumatism, haematuria, fever and other diseases. Powdered seeds are used to massage the teeth and gums to protect from plaque and cavities, as antimicrobial, in treatment of herpes and a medicine for Parkinson's disease and screening was done on antioxidant activity (Vellingiri, Aruna and Hans, 2011) antibacterial activity (Tapas *et al*., 2012) and hepatoprotective activity (Parker and Okwesili, 2010).

Keeping in view the above background and biopotential of plants, present investigation was directed towards evaluating the anti-snake venom activities of ethanol and aqueous extract of *Cassia hirsute* against Indian cobra (*Naja naja*) venom induced toxicity. During a field trip to Pathankot (Punjab, India), traditional healers shared information that root extracts have been in use for snakebite case from generation in their family.

MATERIALS AND METHOD

Collection of Plant and Preparation of Extracts

The plant *Cassia hirsute* were collected from forest near Nelmangala, Dist. Tumkur, State Karnataka and was identified by Dr. M.D. Rajanna, Dept of Botany, GKVK. University of agriculture science, Bangalore. A voucher specimen is been deposited at Museum, Mallige College of Pharmacy, Bangaluru. The roots were cleaned and air dried then subjected to coarse powdering and passed through a sieve # 44 to get uniform powder size. The collected powder was successively extracted with petroleum ether to defat and then by ethanol for 24 hr by using soxhlet apparatus and followed by water using maceration. After the extraction, solvents was distilled off to get concentrated residue and completely dried by lyophilization and stored in air tight container under refrigeration (Shalavadi *et al*., 2013). ECH=Ethanol extract of *Cassia hirsute* and ACH=Aqueous extract of *Cassia hirsute*

Animals

The Wistar rats of either sex (230-260 gm) and Swiss albino mice were used. The animals were housed at room temperature (22-28 ºC) with 65±10% relative humidity for 12 hr dark and light cycle and given standard laboratory feed (Amruth, Sangli, Maharashtra) and water *ad libitum.* The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee.

Chemicals

Tween-80 (Rankem, New Delhi, India), CK Nac kit. (Span Diagnostic Ltd., Surat, India), *Naja naja* venom (Haffkines Institute, Mumbai), Phenobarbitone Sodium, Human Citrated Plasma (K.R. Hospital, Bangalore), Sheep Blood was collected from Srinagar, Bangalore and Phosphate Buffer Solution (PBS) of pH 7.2. All the other solvents and chemicals used for extraction and phytochemical investigation were of analytical grade.

Preparation of Venom Sample

The lyophilized Snake Venom of *Naja naja* was preserved in a desiccator at4 ℃. It was dissolved in PBS of pH 7.2 for further use.

LD_{50} and Minimum Lethal Dose (MLD) of Naja naja Venom

The median Lethal dose (LD50) and minimum lethal dose (MLD) of *Naja naja* venom was assayed by injecting different concentrations of venom in 0.2 ml normal saline into the peritoneum of Wistar albino rats.

Neutralization Activity of *Cassia hirsute* against Lethality Induced by Naja naja Venom (2mg/kg) in Rats

The ability of the ethanol and aqueous extract of *Cassia hirsute* to neutralize lethal toxicity of venom was assessed by *in-vivo* neutralization test. In *in-vivo* method the plant extracts was administered to animals 1 hour prior to challenge with MLD dose of venom to animal. *In-vivo* neutralization was performed in rats (n=6). The rats were administered with ethanol and aqueous extracts (50,100, 200, 300, 400 and 500 mg/kg) of *Cassia hirsute* through oral route one hour prior to administration of 2 mg/kg of MLD of venom by intraperitoneal route; all the animals were observed for mortality for 24 hrs (Abubakar *et al*., 2000).

Phospholipase A_2 Activity

Phospholipase A_2 activity was assessed by indirect hemolytic activity assay on agarose-erythrocyte-egg yolk gel plate as described by Gutierrez *et al*. (1988). 300 µl of packed sheep erythrocytes washed four times with saline solution, 300 µl of 1:3 egg yolk solution in saline solution and 250 µl of 0.01 M $CaCl_2$ solution were added to 25 ml of 1% (w/v) of agar at 50ºC dissolved in PBS pH 7.2. The mixture was applied to Petri dish and allowed to gel. Then, 3 mm diameter wells were made and filled with 15 µl venom samples. After 20 hr of incubation at 37ºC, the diameters of hemolytic halos were measured. To determine the minimum hemolytic dose (MHD) of *Naja naja* venom, 15 µl of solutions containing different amounts of venom concentration (from 1 to 50 µg), were applied into the wells. Control wells contained 15 µl of Phosphate buffer of pH 7.2. After 20 h of incubation at 37ºC, the diameters of hemolytic halos were measured. The minimum hemolytic dose (MHD) was defined as the amount of venom that induced a hemolytic halo of 11-mm diameter (Marunak *et al*., 2007 and Meenatchisundaram, 2008).

Neutralization of phospholipase A_2 activity (indirect hemolytic activity) by ethanolic and aqueous extracts of *Cassia hirsute* was performed by neutralizing venom mixture containing constant amount of [15µg (1 MHD)]

venom incubated with different amounts of ECH and ACH (6.25-800 µg) for 30 min at 37°C. The 15µl aliquots of the mixtures (venom + extracts) were added to wells in agarose-egg yolk-sheep-erythrocytes gels, plates were incubated at 37°C for 20 h. Control samples [15µg (1 MHD)] contained venom without extract. Plates were incubated at 37°C for 20 hr. Neutralization was expressed as the % Inhibition that reduced 50% the diameter of the hemolytic halo when compared to the effect induced by venom alone.

Coagulation Activity

The coagulation activity of the ECH and ACH against the anticoagulant response caused by*Naja naja* venom by described by Theakston *et al*. (1983). Assay was started by finding minimum coagulation dose of plasma (MCD-P) of venom and assayed by taking various concentration of venom (1-150 µg), dissolved in 100 µl of PBS of pH 7.2 and to this 300 µl of human citrated plasma was added which was collected from Blood Bank of K.R.Hospital, Bangalore, India; to this 100µl of 0.25M $CaCl_2$ was added and the clotting time was observed every 15 sec by gentle tilting until coagulation occurred. Plasma incubated with PBS alone served as control. MCD-P was defined as the least amount of venom that clots plasma in 60 sec at 37°C (Karlsson and Pongsawasdi, 1980).

The neutralization of anticoagulation activity of ECH and ACH induced by *Naja naja* venom was done by taking constant amount of venom (2 MCD-P) which was mixed with various concentrations of ECH (50-350µg) and ACH (50-300 µg). The mixtures were incubated for 30 min at 37°C and then 100µl of mixture was added to 300 µl of citrated plasma, the plasma was recalcified with the addition of 100 µl of 0.25 M $CaCl_2$ and the clotting times were recorded by gentle tilting at every 15 sec till coagulation occurred.

Effect of ECH and ACH on Naja naja Induced Bleeding

Bleeding time test was performed using male Swiss albino mice weighing 18-20gm. The mice were grouped in 8 groups, each group contained 6 animals and group 1 received vehicle orally, group 2 received vehicle orally and *Naja naja* venom LD_{50} dose by i.v route and group 3 to 8 received required doses of ECH (300, 600 and 900 mg/kg) and ACH (300, 600 and 900 mg/kg) by oral route one hour before the administration of *Naja naja* venom (i.v. route). After one hour all the animals were anaesthetized with Ketamine (45 mg/kg i.p. route). A wound was made on the tail vein of the mouse by surgical razor at 5 cm from the tip. The blood was absorbed on Whatman no.1 filter paper at every 15 sec interval till no blood stain was appeared on the filter paper then total bleeding time was recorded (Karasudani *et al*., 1996).

Edema-forming Activity

The Minimum edema-forming dose (MED) of *Naja naja* venom was determined by the method of Camey *et al*. (2002).The Minimum edema-forming dose was defined as the least amount of venom which when injected subcutaneously into rat footpad results in 30% edema within 6 hours of venom injection. The volume of each footpad was measured every 60 min after venom injection with a digital plethysmometer. The rats were grouped in 8 groups, each group containing 6 animals and group 1 received vehicle orally, group 2 received vehicle orally and intraplantar route *Naja naja* venom (6µg/100µl) and group 3 to 8 received required doses of ECH (100, 200 and 300 mg/kg) and ACH (100, 200 and 300 mg/kg) by oral route one hour prior to the administration of *Naja naja* venom by intraplantar route. One hour after venom injection change in the paw volume was recorded every hour till completion of 5 hours.

Statistical Analysis

All the data are expressed in mean ± SEM. The significance of differences in means between control and treated animals for different parameters was determined by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. The minimum level of significance was fixed at $p<0.05$.

RESULTS

LD_{50} and MLD of Naja naja Venom

The table 1 reveals that the results of lethality tests on *Naja naja* venom for determination of median lethal dose (LD_{50}) and the minimum lethal dose (MLD) after intraperitoneal injection in rats. The median lethal dose (LD_{50}) of the venom was 1.5mg/kg and minimum lethal dose (MLD) was found as 2mg/kg.

Table 1: LD_{50} and Minimum lethal dose (MLD) of *Naja naja* venom

Groups	Venom Dose (mg/kg)	No. of deaths/ No. of rats used (within 24 hrs of venom administration)	% Death
1	-	0/6	0
2	0.5	0/6	0
3	1	1/6	16.67
4	1.5	3/6	50
5	2	6/6	100
6	2.5	6/6	100

Neutralization Activity of *Cassia hirsute* against Lethality Induced by Naja naja Venom (2mg/kg) in Rats

Naja naja venom induced lethality was significantly antagonized by both ECH and ACH. Both extracts at 400mg/kg dose shown 66.67% survival and effective dose 50% (ED_{50}) of ECH and ACH after intraperitoneal injection in venom pretreated rats were found to be 200mg/kg and 300mg/kg body weight respectively and results were summarized in table 2.

Table 2: *In vivo* Neutralization activity of *Cassia hirsute* against lethality induced by *Naja naja* venom (2mg/kg) in rats

Groups	Dose (mg/kg) extract	Mortality (within 24h of venom +extract administration [No. of death/ No. of rats used]	% Survival (24h)
ECH	0	6/6	0
	50	6/6	0
	100	5/6	16.67
	200	4/6	33.33
	300	3/6	50
	400	2/6	66.67
	500	2/6	66.67
ACH	0	6/6	0
	50	6/6	0
	100	6/6	0
	200	5/6	16.67
	300	3/6	50
	400	2/6	66.67
	500	2/6	66.67

Phospholipase A_2 Activity

In present study *Naja naja* venom was able to produce hemolytic haloes in agarose-sheep erythrocytes gels because venom have the enzyme phospholipase A_2 that has the ability to lyse sheep RBC's. About 15 µg of *Naja naja* venom produced 11.26 mm diameter haemolytic haloes (Table 3) thus 15µg *Naja naja* venom was taken as minimum hemolytic dose (MHD). Both ECH and ACH inhibited phospholipase A_2 dependent haemolysis of sheep RBC's induced by *Naja naja* venom in a dose dependent manner. From table 4 and 5, it was found that 800µg of ECH inhibited haemolysis by 56.66% with 4.88mm haloes but with ACH it was 82.24% with 2mm haloes.

Table 3: Phospholipase A_2 [*Naja naja* venom] induced hemolysis on sheep RBC

Venom (µ gm)	Haloes (mm)
1	02.55
3	04.60
6	06.50
9	08.57
12	10.09
15	11.26
18	13.08

Table 4: Effect of ECH and ACH on Phospholipase A_2 (*Naja naja* venom) induced hemolysis of sheep RBC

Venom (15 µg)+ ECH (µg)	Haloes (mm)	Inhibition (%)
6.25	10.92	3.02
12.5	10.02	11.01
25	9.48	15.81
50	8.98	20.25
100	8.32	26.11
200	7.04	37.48
400	6.82	39.43
800	4.88	56.66

Table 5: Effect of ACH on Phospholipase A_2 [*Naja naja* venom] induced hemolysis on sheep RBC

Venom (15µg) + ACH(µg)	Haloes (mm)	Inhibition (%)
6.25	10.02	11.01
12.5	9.80	12.97
25	8.62	23.45
50	7.36	34.64
100	6.92	38.54
200	5.02	55.42
400	4.12	63.41
800	2.00	82.24

Coagulation Activity

Naja naja venom was found to be anti-coagulant in nature hence; the coagulation activity ECH and ACH was performed using the human plasma. Minimum coagulation dose of plasma (MCD-P) of *Naja naja* venom was found to be 60 µg. To test the coagulant activity of the ECH and ACH the 2 X MCD-P was used i.e., 120 µg of venom. 120 µg was kept constant and varying doses of extracts i.e ECH (50-350µg) and ACH (50-300µg) were used. In control (venom treated) the coagulation time was found to be 121.5±2.172sec which was increased significantly (P<0.001) as compared with normal coagulation time was produced (Plasma + PBS+ CaCl2) at a time period of 23.83±0.307 sec. In contrast, the ECH (100, 150, 200, 250, 300 and 350 µg) and ACH (100, 150, 200, 250 and 300 µg) treated showed a high significant reduction (P<0.001) in the anti-coagulant activity of venom with 80.51% inhibition of anti-coagulation of ECH at 350 µg and 79.84% of ACH at 300 µg and results were summarized in table 6.

Table 6: Effect of ECH and ACH on *Naja naja* induced anticoagulation (Coagulation activity)

Group	Dose of venom(120 µg)/varying dose of ERS in µg	No. of folds	Formation of clot Mean ± SEM (Seconds)	% Inhibition of anti-coagulation {Formation of clot by venom[sec] - formation of clot by extract [sec] ÷ Formation of clot by venom[sec]} X 100
Normal	PBS+$CaCl_2$	-	23.83±0.307	-
Venom	120		121.5±2.172[a]	-
Venom + ECH + $CaCl_2$	50	2	113.5±1.455**	6.58
	100		96.33±1.333***	20.71
	150		73.67±1.282***	39.36
	200		56.67±1.978***	53.35
	250		29.83±1.400***	75.44
	300		27.50±0.922***	77.36
	350		23.67±0.715***	80.51
Venom + ACH + $CaCl_2$	50	2	116.8±2.197	3.86
	100		89.17±2.301***	26.6
	150		63.50±1.335***	47.73
	200		38.83±1.797***	68.04
	250		25.33±1.256***	79.15
	300		24.50±1.176***	79.84

Effect of ECH and ACH *on Naja naja* Induced Bleeding

Table 7 reveals that the bleeding time significantly (P<0.001) increased in venom treated group i.e. in first hour it was 207.5±4.61 sec as compared with normal group. In contrast the ECH 300, 600 and 900 mg/kg shows significant (P<0.001) decrease in bleeding time 162.5±4.610, 145.0±3.162 and 137.5±4.610 sec respectively in first hour as compared with venom treated group. Similarly ACH 300, 600 and 900 mg/kg also shown significant (P<0.001) decrease in bleeding time 175.0±3.162, 152.5±4.610 and 152.5±4.610 sec respectively as compared to venom treated group.

Table 7: Effect of ECH on *Naja naja* induced bleeding

Bleeding time in hours	Groups							
	Normal	Venom control	ECH 300 mg/kg		ECH 600 mg/kg		ECH 900 mg/kg	
	Bleeding time (Sec)	Bleeding time (Sec)	Bleeding time (Sec)	% Inhibition	Bleeding time (Sec)	% Inhibition	Bleeding time (Sec)	% Inhibition
1	102.5±6.021	207.5±4.610[a]	162.5±4.610***	21.68	145.0±3.162***	30.12	137.5±4.610***	33.73
2	100.0±5.000	202.5±3.354[a]	152.5±2.500***	24.69	140.0±3.162***	30.86	130.0±3.162***	35.8
3	97.50±5.123	185.0±6.325[a]	155.0±3.162***	16.21	142.5±3.354***	22.97	135.0±3.873***	27.07
4	95.00±3.162	185.0±6.325[a]	152.5±2.500***	17.56	145.0±5.000***	21.62	132.5±2.500***	28.37

% Inhibition of bleeding time= {[Bleeding time of Venom control- Bleeding time of Treated group]÷ Bleeding time of Venom control**}** X 100

Edema-forming Activity

Anti-inflammatory activity of ECH and ACH against venom induced paw thickness was carried out in rats. In edema forming activity, the rats injected with *Naja naja* venom showed increase in paw thickness. About 6 µg of venom induced more than 30% edema formation within 3 hrs which is considered as 100% activity and it was considered as MED. The venom treated control animals shown significant (P<0.001) increase in paw edema in 2ndhr and onwards as compared with normal group. In contrast animals pre-treated with ECH and ACH (100, 200 and 300 mg/kg) one hour before challenging with MED of venom showed a highly significant (*P*<0.001) decrease in paw edema at 3rd, 4th and 5th hr as compared with control treated animals. The 50% inhibition was shown by both extracts at 2ndhr and result was summarized in table 8.

Table 8: Effect of ACH on Naja naja induced bleeding

Bleeding time in hours	Groups							
	Normal	Venom control	ACH 300 mg/kg		ACH 600 mg/kg		ACH 900 mg/kg	
	Bleeding time (Sec)	Bleeding time (Sec)	Bleeding time (Sec)	% Inhibition	Bleeding time (Sec)	% Inhibition	Bleeding time (Sec)	% Inhibition
1	102.5±6.021	207.5±4.610[a]	175.0±3.162***	15.66	152.5±4.610***	26.50	152.5±4.610***	26.50
2	100.0±5.000	202.5±3.354[a]	167.5±4.610***	17.28	147.5±2.500***	27.16	140.0±5.000***	30.86
3	97.50±5.123	185.0±6.325[a]	170.0±3.162	8.10	152.5±4.610***	17.56	142.5±6.423***	22.97
4	95.00±3.162	185.0±6.325[a]	167.5±2.500*	9.45	150.0±3.873***	18.91	142.5±6.423***	22.97

% Inhibition of bleeding time= {[Bleeding time of Venom control- Bleeding time of Treated group]÷ Bleeding time of Venom control**}** X 100

Table 9: *Naja naja* venom –induced odema and its inhibition by ethanol and aqueous extracts of *Cassia hirsute*

Treatment	0 h	1st h		2nd h		3rd h		4th h		5th h	
	Paw Volume (ml)	Paw Volume (ml)	% Inhibition	Paw Volume (ml)	% Inhibition	Paw Volume (ml)	% Inhibition	Paw Volume (ml)	% Inhibition	Paw Volume (ml)	% Inhibition
Normal	0.035±0.002	0.052±0.005	-	0.032±0.0008	-	0.0315±0.0052	-	0.030±0.0047	-	0.027±0.00445	-
Control	0.040±0.001	0.135±0.032	-	0.29± 0.038[a]	-	0.265±0.009[a]	-	0.3283±0.026[a]	-	0.362±0.015[a]	-
ECH (100 mg/kg)	0.097±0.026	0.12±0.021	11.11	0.098±0.0033	66.20	0.086±0.0032***	67.54	0.069±0.0047***	78.98	0.065±0.0049***	82.04
ECH (200 mg/kg)	0.063±0.011	0.15±0.014	11.11	0.087±0.004	70	0.062±0.0019***	76.60	0.052±0.0026***	84.16	0.048±0.001***	86.74
ECH (300 mg/kg)	0.13±0.023	0.16±0.012	18.51	0.078±0.0028	26.89	0.059±0.0022***	77.73	0.045±0.0028***	86.29	0.0435±0.0034***	90.33
ACH (100 mg/kg)	0.13±0.036	0.11±0.038	18.51	0.095±0.0040	67.24	0.0808±0.003***	69.90	0.063±0.0015***	80.81	0.0645±0.0018***	82.18
ACH (200 mg/kg)	0.037±0.003	0.14±0.029	3.70	0.083±0.0035	71.37	0.0713±0.0021***	73.09	0.044±0.0034***	86.59	0.041±0.0012***	88.67
ACH (300 mg/kg)	0.17±0.043	0.19±0.056	40.74	0.069±0.0125	76.20	0.062± 0.0021***	76.60	0.0465±0.0063***	85.83	0.044±0.006***	87.84

DISSCUSSION

Snake envenomation causes pathological changes in the victims, ultimately leading to death in the absence of proper medical aid. Several pharmacological actions exhibited by the venoms are due to the combined action of various site-specific toxins and hydrolytic enzymes, whose concentrations and potencies are known to vary, depending upon various factors (Chippaux, Williams and White, 1991) thus complicating the management of snake bite. Many Indian medicinal plants are recommended for the treatment of snakebites (Alam and Gomes, 2003). Both ethanol and aqueous extracts of *Cassia hirsute* significantly inhibits the lethality induced by the *Naja naja* venom. The ACH contains glycosides which may be responsible of antivenom activity as it is mentioned in literature that glycosides are found widely in anti-snake venom plants and provide nearly 20% protection against snake venom (Argiolas and Pisano, 1983) it also contains β-sitosterols which is the most abundant of the phytosterols which might have neutralized toxic enzymes of the *Naja naja* venom. The obtained results are in-line with literature that suggests that plant *Pluchea indica* containing β-sitosterols neutralizes the cobra venoms toxic reactions (Gomes *et al*., 2007).

Rupturing of blood capillaries of tissues was necessary for the release of haemoglobin and it may be contributed by phospholipid hydrolysis and protease activity present in *Naja naja* PLA_2 (Datta and Bhattacharyya, 1999). Some constituents of plants have the ability to bind proteins and inhibit their enzymatic activity as well as the catalytic activity of snake venoms PLA_2 (Gowda, 1997 and Melo and Ownby, 1999). As *Cassia hirsute* contains tannins as well as β-sitosterols, glycoside which have the ability to bind with the proteins that produces toxic effects, in this study the different concentrations of ECH and ACH were evaluated for their activity against PLA_2. Both ECH and ACH displayed significant inhibition of PLA_2 activity.

Increase in concentration of *Naja naja* venom prolongs the clotting time of human plasma. The anticoagulant effect of PLA_2 from *Naja naja* venom is due to enzymatic degradation of phospholipids necessary for the adherence and activation of coagulation protein factors. Incubation of ECH and ACH is able to prevent the anticoagulation action by preponing coagulation time against *Naja naja* venom. The neutralization of anticoagulation might be due to active component of *Cassia hirsute*. *Naja naja venom* showed more prolonged bleeding time from that of the heparin, which indicates that the *Naja naja* venom contains potent anti-coagulant property. The ECH and ACH have shown high significant activity as compared to control group.

As the main component of the *Naja naja* venom is PLA_2 which also has characteristic activity of producing edema, development of edema is a common feature of the coetaneous inflammatory response and is dependent on a synergism between endogenous mediators that increase vascular permeability and those that increase blood flow (Ketelhut *et al*., 2003). Both extracts showed significant reduction in paw thickness as compared to venom control group. This is due to the presence of steroids and terpenoids in the extracts (Zeashan, 2009).

CONCLUSIONS

In conclusion, these findings suggest a potential antivenom role of *Cassia hirsute* against *Naja naja* venom. Further studies are required to pursue the interesting lead emerging from the present results to exploit the full therapeutic potential of *Cassia hirsute*, as antivenom.

Conflicts of Interest

There is no conflict of interest.

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