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Preliminary studies of antivenom and antioxidant activities of *Gymnema sylvestre* R.Br. leaf extracts.

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Medicinal plants are rich in biologically active phytoconstituents and therapeutic compounds that have wide applications in pharmaceutical industry. The ameliorative actions of such phytochemicals could be attributed to the presence of dietary fibre, detoxifying mediators, neuropharmacological agents, antioxidants, anticancer mediators, *etc.* In the present study, we investigated the *in vivo* antivenom potential of *Gymnema sylvestre* R. Br leaf extract (GSE)in experimental animal model. The study also investigated the antioxidant potential (*in vitro)* of GSE (aqueous and methanolic) along with qualitative phytochemical constituent tests. Phytochemical tests were performed by qualitative method to detect the presence of different phytoconstituents within the leaf. Different quantitative tests (DPPH scavenging, H_2O_2 scavenging, Ferric reducing activity) were performed. The IC_{50} value was determined from the different concentration. To detect the venom neutralization effects, *in vitro* PLA_2 and *in vivo* lethality, haemorrhage, edema neutralization studies were performed. Aqueous and methanolic extracts of the leaf (400 mg/kg and 100 mg/kg body wt.*)* provided protection against the lethal dose of venom and showed a successful anti-hemorrhagic and edema neutralization activity against *Daboia russelli* venom (DRV). *In vitro* PLA_2 neutralization activity of the leaf showed up to 3-fold protection. Methanolic extract exhibited the significant results in both qualitative phytochemical analysis as well as *in vitro* antioxidant studies. The results specify that the leaves of *Gymnema sylvestre* R.Br. possess antioxidant as well as antivenom potential and could act as a free radical inhibitor

Keywords: Ayurvedic, *Gurmar*, Herbal, Periploca of the woods

In the present decade, there has been an increasing interest in naturally occurring herbal compounds. Plants may possess safe, positive therapeutic potential as antioxidants and can be tested through phytochemical and biological screening[1]. The naturopathic treatment of diseases has been extensively investigated from ancient times. *Gymnema sylvestre* (Asclepiadaceae), locally known as "*Gurmar*" is a well-known herb in Ayurvedic system of medicine. The phytoconstituents in the leaf extract contains sweet suppression activity including triterpene saponins like gymnemic acid, gymnema saponins and polypeptide gurmarin. This herb hosts a plethora of therapeutic effects including its use in treatment of diabetes[2], arthiritis, asthma and as anti-inflammatory agent.

Snakebite is one of the major and commonly fatal socio-medical issue, affecting the public health specifically in remote areas of tropical countries[3]. The World Health Organization (WHO) approximates that there are nearly 5.4 million snake stings and 1.8-2.7 million cases of envenomation with 81,410-1,37,880 loss of life each year globally[4]. Cobra (N*aja naja*), Russell's viper (*Daboia russelli*), Krait (*Bangarus caeruleus*), and Saw Scaled Viper (*Echis carinatus*) are most typical ferocious snakes found in the dense tropical regions of India[5]. Being hemotoxic in nature the viperine venoms of *Daboia russelli* (Shaw & Nodder) belonging to *Viperidae* family mainly affect the blood circulation causing intra vascular hemolysis and depression of the coagulation mechanism as well as producing changes in the nervous system or in vascular dynamics. Venoms of different species are a mixture of toxins and enzymes in varying proportion having toxicity and antigenic structure. The enzymatic components lead to systematic and local effects and the non-enzymatic ones provide lethality[6].

The conventional treatment for snakebite is antivenom which has many side effects and related complication[7]. In this context, variety of plants are extensively studied for their antivenom activity.

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Earlier snake venom was inhibited successfully by certain plant extracts such as *Andrographis paniculata* and *Aristolochia indica*[8].

Gymnema sylvestre R.Br., *the* large, woody climber, more or less pubescent, belongs to the family Asclepiadaceae[9]. *Gymnema* leaf extract, notably the peptide 'Gurmarin', has been found to interact the taste buds on the tongue to taste sweet and bitter. Gymnemic acid has a similar effect. The leaves of the plant have hypolipidemic activity. It is widely grown throughout India and other tropical and sub-tropical regions of the globe. It is used as a traditional medicinal plant with various folkloric claims. Having variety of therapeutic activities, it has been valued in *Ayurveda* for medication[10,11]. This plant is known to possess propitious effects as digestive, anti-inflammatory, diuretic, hypoglycemic and antihelmentic[12,13.] In the present study, we evaluated the presence of different types of phytochemicals, as well as antioxidant and venom neutralization property of leaf extract of *Gymnema sylvestre* R.Br. (GSE).

Materials & Methods

Snake venom and snake venom antiserum

Commercially purchased snake venom was used for this study. DRV (*Daboia russellii venom)* was procured from Irula Snake Catcher's Cooperative Society (ISCICS), Kancheepuram, Chennai, India & dissolved in 0.9% *w/v* NaCl, spinned at 2,000 rpm (revolution per min) for 10 min. Lyophilized polyvalent snake venom antiserum I.P (batch no. 4066016) was obtained from Bengal Chemical Pharmaceutical *Pvt.* Ltd., Calcutta, India.

Animals

Swiss albino mice (Male) weighing 20±2 g were procured from local supplier and sustained in optimum laboratory conditions. They were provided standard laboratory diet and water *ad libitum*. The University Animal Ethics Committee, Department of Physiology, Vidyasagar University, Paschim Medinipur, India approved all animal experiments. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India (Clearance no. VU/IAEC/CPCSEA/5/9/2024) was followed. The animals were allocated as follws: Group wise animal allocation was as follows: Groups (n=6); Group I was saline control (0.9% w/v); Gr. II, venom control (DRV); Gr. III, venom + aqueous leaf extract; and Gr. IV, venom + methanolic leaf extract. n: Number of animals in each group.

Collection and preparation of aqueous and methanolic leaf extract

The plant materials of *Gymnema sylvestre* R.Br. were locally obtained from Indigenous Crude Drugs Seller, Kolkata-700 007, India. The collected *Gymnema sylvestre* R. Br. leaf was identified by the taxonomist of Vidyasagar University, and was shade dried for 72 h and was grinded to get fine powder. 2000 mg of leaf powder was added to 10 mL of distilled water and was dissolved for 24 h. After that the extract was filtered by Whatman No. 1 filter paper and collected in plastic bottle and stored at 4ºC for further experimentation. To prepare methanolic extract, 10 g of powdered *Gymnema sylvestre* R. Br. leaf was dissolved in 25 mL methanol for extraction of solvent for 24 h. Whatman No.1 filter paper was used to filter the extract and air-dried filtrate was kept for 4-5 days. The powder was stored for making different concentration of methanolic leaf extract. The moisture content of the extracts before and after weighing was 15%.

Qualitative phytochemical analysis

Freshly prepared leaf extracts were used for primary phytochemical screening to assess the presence of the following phytoconstituents; alkaloids[14], cardiac glycosides[15], quinoes[15], tannins[15], phenols[14], saponins[15], flavonoids[14], reducing sugar[15] and protein[14]. Standard procedure was used for qualitative assessment[14,15].

In vitro antioxidant activity of *Gymnema sylvestre* R.Br. leaf extract

Scavenging activity of DPPH radical

Free radical scavenging activity was studied by DPPH assay of the extract as per the method given by Blois[16]. About 0.5 mL of aqueous and methanolic leaf extracts of GSE (2, 4, 8 and 16 mg/mL), 3 mL absolute ethanol and 0.3 mL of ethanolic solution of DPPH were placed in the test tube. The mixture was kept in the dark at room temperature for 10 min and absorbance was measured at 517 nm against a blank. The same procedure was used for ascorbic acid as standard. The given equation was adopted to calculate the proportion of the radical scavenging activity of each extract. IC_{50} is defined as the concentration of extract used to decrease activity by 50% (IC_{50}) under the specified experimental condition.

$$\text{DPPH radical scavenging effect (\%)} = 100 - \left(\frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of control}}\right) \times 100$$

Free radical scavenging activity (*FRSA*) *using hydrogen peroxide*

Czochra & Widensk[17] was used to assess the hydrogen peroxide FRSA activity of aqueous and methanolic leaf extract. 2 mL of hydrogen peroxide (43 mM) and 1.0 mL of GSE extract (2, 4, 8 and 16 mg/mL) followed by 2.4 mL of 0.1 M phosphate buffer (pH 7.4) were added. The solution was kept for 10 min and the absorbance was recorded at 230 nm. All reading were repeated thrice. Blank was prepared without adding H_2O_2 and control was prepared without sample. The FRSA %was calculated as

FRSA (%) = $[(V_0 – V_1)/ V_0] \times 100$

where, V_0 = Absorbance of control and V_1= Absorbance of sample

Scavenging effect (%) = $100 \times (A_0 – A_S) / A_0$

where A_0 is the absorbance of the blank and A_S is the absorbance of the sample.

Determination of ferric reducing property

The reducing property of the extract was determined by the method of Maruthamuthu & Kandasamy[18]. One mL of the aqueous and methanolic extract of GSE (2, 4, 8 and 16 mg/mL) was mixed with 2.5 mL of 0.2 M of sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Both the mixtures were incubated at 50ºC for 20 min thereafter 2.5 mL of 10% TCA was also added and centrifuged for 10 min at 3000 rpm. About 2.5 mL of supernatant was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% of $FeCl_3$. Absorbance of the solution was measured at 700 nm.

Acute toxicity

An acute toxicity test was accomplished using the guideline of the Organization of Economic Co-operation and Development (OECD) guideline 420 for testing of chemicals[19]. Male and female swiss albino mice, aged 6–8 weeks were used. Various concentration of aqueous and methanolic *Gymnema sylvestre* R.Br. leaf extract (GSE) was administered intra peritoneally for 14 days (n=6). After administration of GSE, mice were kept under observation for 24 h and observed. The animals were monitored for 14 days. The mice were weighed and visual observations for mortality, changes in physical appearance, behavioral pattern (lethargy, salivation, fur and sleep), injury, pain, and signs of illness were noticed during the period.

***In vivo* neutralization studies of snake venom (DRV)**

Minimum lethal action

Varied concentrations of DRV were injected (0.1 mL) intravenously in male albino mice to evaluate the minimum lethal dose (MLD) of the venoms (*Daboia russelli* venom-DRV) and the number of mortalities were noted[20]. To estimate the neutralization of lethal action of crude leaf extracts of *Gymnema sylvestre* R.Br. (GSE), Various concentration (200, 500, 1000 and 2000 mg/kg body wt.) of leaf extract was mixed with lethal dose of venoms (DRV) (2 µg) and incubated at 37ºC for 60 min and centrifuged for 10 min at 2000 rpm. Supernatant was collected and intravenously injected.

Haemorrhagic activity

The Minimum Hemorrhagic Dose (MHD) was evaluated for venom as the amount of venom that produced a haemorrhagic halo of 10 mm after 24 h via intradermal injection. Haemorrhage neutralization activity was evaluated by incubating MHD of venom (10 µg) with aqueous (200 mg/kg body wt.) and methanolic (50 mg/kg body wt.) GSE. Incubation of the mixture was done at 37°C for 1 h and 0.1 mL of the mixture was injected intradermally into mice. The hemorrhagic lesion was estimated after 1 day [20].

Edematic activity (*MED*)

Minimum edema dose (0.01 mL) of venom was injected intradermally in mice and edematous action of venom was observed for successive 4 h[21]. Edema neutralization activity was assessed by injecting 200 mg/kg body wt. of aqueous extract and 50 mg/kg body wt. of methanolic GSE extract with minimum edema dose(1 µg) of venom and changes were observed up to 4 h. This was followed by measurement of edema with digital caliper.

Defibrinating activity (*MDD*)

The least dosage of venom which produces incoagulable blood in mice within 1 h is defined asMinimum defibrinating dose (MDD). The MDD of venom (1.5 µg) was provided to the mice (20±2 g) *in vivo* through intravenous injection. Later in the *in vivo* antagonism by GSE, the extract was incubated (100 mg/kg body wt. for aqueous and 20 mg/kg body wt. for methanolic extracts) with venom (at 37ºC for 60 min) centrifuged at 2,000 rpm for 10 min.

***In vitro* neutralization studies of snake venom**

Phospholipase A_2 activity

The *Daboia russelli* venom's phospholipase A_2 unit (PLA_2) (0.1 µg) was assessed by egg yolk coagulation method[22] where one unit of enzyme activity is measured by the increasing the egg yolk coagulation time by 1 min.

Table 1 — Result of phytochemicals tests

Phytochemicals	Tests	Observation	Aqueous extract	Methanolic extract
Tannin	$FeCl_3$ Test	Green or blue colour	+	++
Saponin	Frothing Test	Persistence of stable froth	+	+++
Alkaloid	Wagner's Test	Development of reddish-brown precipitate or colour	+	++
Flavonoid	Alkaline Reagent Test	Manifestation of intense yellow colour	+	++
Quinone	HCl Test	Formation of red colour	-	++
Phenol	Mayer's Test	Manifestation of white precipitate	+	-
Glycosides	Fehling Test	Brick red precipitation formation	+	++
Reducing sugar	Fehling Test	Dark brick red colour	+	++
Protein	Adamkiewicz Test	Purple colour develops at the junction of the liquid	+	++

Statistical analysis

Results were provided as Mean ± SE and analysed by one-way ANOVA. Differences were considered as statistically significant at $P<0.05$ as compared to control(s).

Results

***In vitro* phytochemical constituents' study of aqueous and methanolic extract of *Gymnema sylvestre* R.Br.** (GSE)

Phytochemical screening are primary tests used to detect the presence of primary and secondary metabolites in the leaf extracts. Several qualitative analyses with observation are mentioned below to detect the presence of the different phytochemicals. Observations of the phytochemical tests are provided in Table 1.

***In vitro* antioxidant activity of aqueous and methanolic extract of** GSE

The *in vitro* antioxidant activity *i.e.*, DPPH scavenging activity, hydrogen peroxide and reducing activity at various concentration (2-16 mg/mL) of plant extracts (aqueous and methanolic) was obtained from the calibration curves plotted.

DPPH free radical scavenging activity

Gymnema sylvestre R. Br. (GSE) aqueous extract showed 16.71%, 27.09%, 41.76%, 65.99 % inhibition at a concentration of 2, 4, 8 and 16 mg/mL concentration, respectively. On the other hand, for methanolic extract the percentage inhibition was 22.2, 34.42, 63.96 and 80.05% for the same concentrations (Fig. 1). Both aqueous and methanolic extracts provided significant DPPH radical scavenging activity as compared to ascorbic acid. The IC_{50} value of aqueous extract was 3.24 mg/mL (Fig. 2A) and for methanolic extract it was 2.49 mg/mL (Fig. 2B).

Ferric reducing antioxidant power assay

According to this method the reduction of Fe^{3+} to Fe^{2+} is determined by measuring the absorbance of the solution. The comparison was made between aqueous extract, methanolic extract and standard Ascorbic acid. The mean difference of OD value between aqueous and methanolic extract of *Gymnema sylvestre* R.Br. extract (GSE) was found significant for 2, 4, 8 and 16 mg/mL concentration (Fig. 3A).

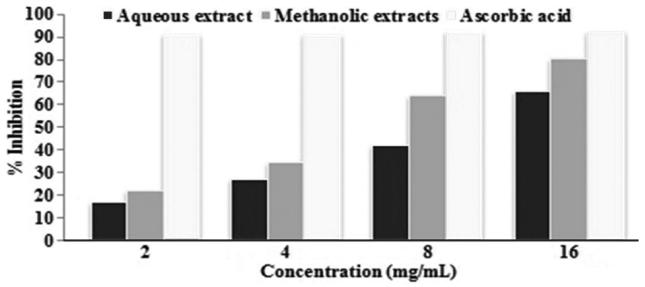

Fig. 1 — DPPH radical scavenging activity of methanolic and aqueous extract of *Gymnema sylvestre* R. Br. (GSE) leaf along with ascorbic acid

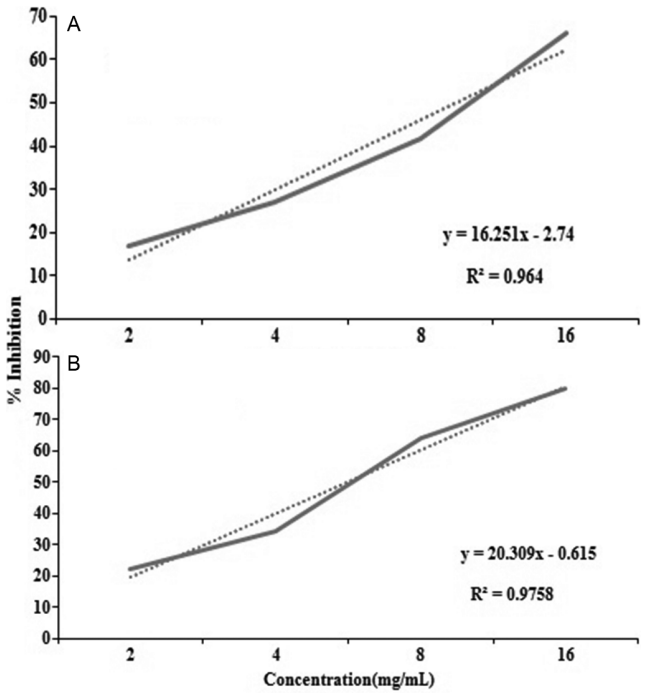

Fig. 2 — (A) IC_{50} (3.24 mg/mL); and (B) IC_{50} (2.49 mg/mL) of Methanolic extract of *Gymnema sylvestre* R. Br. (GSE)

H_2O_2 scavenging activity

Gymnema sylvestre R.Br. at various concentration (2, 4, 8 and 16mg/mL) of leaf extract were compared with ascorbic acid. For aqueous extract of GSE, the percentage inhibition was 13.31, 29.45, 66.05 and 79.53%, respectively for the above concentrations. For methanolic extract of GSE, the percentage inhibition was 28.28, 50.58, 70.71 and 81.69%, respectively (Fig. 3B). The IC_{50} value of aqueous extract was 6.4 mg/mL (Fig. 4A) and for methanolic extract it was 4 mg/mL (Fig. 4B).

Neutralization of lethal dose

The lethal action of *Daboia russelli* venom (DRV) was neutralized by *Gymnema sylvestre* R.Br. leaf extract (GSE) and assessed by injecting (*i.v.*) 2 µg of DRV along with 100, 200 and 400 mg/kg body wt. aqueous *Gymnema sylvestre* R.Br. leaf extract (GSE) and 20, 50 and 100 mg/kg body wt. methanolic GSE. The aqueous and methanolic leaf extract of GSE showed up to 2-fold protection against DRV at a dose of 400 and 100 mg/kg body wt., respectively (Table 2).

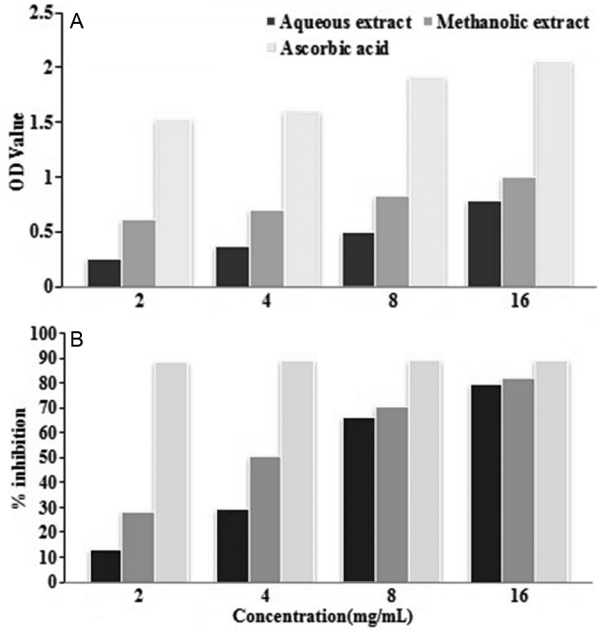

Fig. 3 — (A) Ferric reducing activity; and (B) H_2O_2 radical scavenging activity of aqueous and methanolic extract of *Gymnema sylvestre* R. Br. (GSE) along with ascorbic acid.

Haemorrhage activity

DRV when injected alone showed a hemorrhagic lesion of approximately 13 mm in size (Fig. 5 and Fig. 6A). When DRV was injected along with 200 mg/kg body wt. aqueous GSE the hemorrhagic lesion was found to be 5 mm in size (Fig. 6B). When 50 mg/kg body wt. methanolic GSE was injected, it completely neutralized the venom effect (Fig. 6C).

Neutralization of edema activity

DRV (1 µg) along with aqueous leaf extract (200 mg/kg) and methanolic leaf extract (50 mg/kg) was injected (*i.d.*) and observed the changes in 0, 0.5, 1, 2, 3 & 4 h (Table 3). There was a significant time and dose dependent decrease in Gr. III and Gr. IV as compared to Gr. II (Fig. 7 and 8).

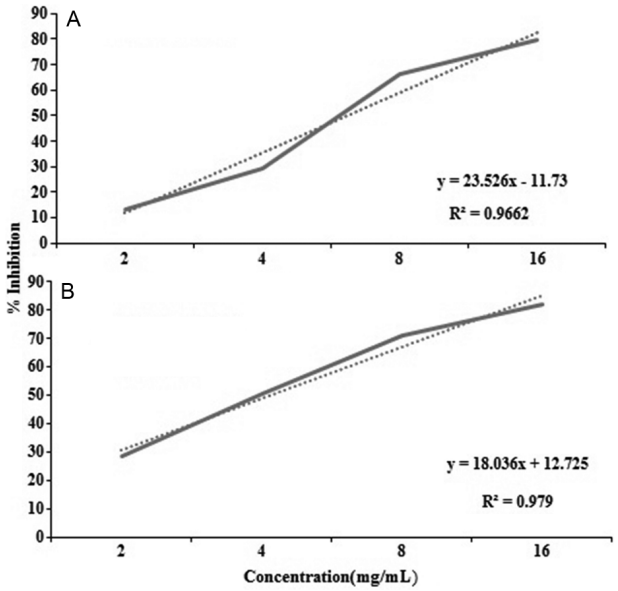

Fig. 4 — (A) IC_{50} (6.4 mg/mL) of aqueous extract; and (B) IC_{50} (4 mg/mL) of Methanolic extract of *Gymnema sylvestre* R. Br. (GSE).

Table 2 — Neutralization of *Daboia russelli* venom activity by aqueous and methanolic extract of *Gymnema sylvestre* R. Br. (GSE)

	Venom + GSE (aqueous)	Venom dose (p/n)	Fold of protection	Venom + (GSE) (methanolic)	Venom dose (p/n)	Fold of protection
Lethal action	DRV(2 µg) + GSE (100 mg/kg body wt.)	2 (6/6)	NP	DRV (2 µg) + GSE (20 mg/kg body wt.)	2 (6/6)	NP
	DRV(2 µg) + GSE (200 mg/kg body wt.)	2 (0/6)	1 Fold (P)	DRV (2 µg) + GSE (50 mg/kg body wt.)	2 (0/6)	1 Fold (P) 1.5 Fold (P)
	DRV(2 µg) + GSE (400 mg/kg body wt.)	2 (0/6)	1 Fold (P) 1.5 Fold (P) 2 Fold (P)	DRV (2 µg) + GSE (100 mg/kg body wt.)	2 (0/6)	1 Fold (P) 1.5 Fold (P) 2 Fold (P)

NP, Not protected; P, Protected; DRV, *Daboia russelli* venom; GSE, *Gymnema sylvestre* R.Br. leaf extract (GSE); (n=6); n, Number of animals in each group.

Neutralization of defibrination activity

Venom induced defibrination action was neutralized by 100 mg/kg body wt. aqueous extract and 20 mg/kg body wt. methanolic GSE. The leaf extract of GSE gave up to 1.13-fold protection against viper venom induced defibrination (Table 4).

Neutralization of PLA_2 activity

In the *in vitro* PLA_2 studies with *Gymnema sylvestre* R.Br. both aqueous and methanolic extracts of GSE showed up to 3-fold protection against DRV (Table 5).

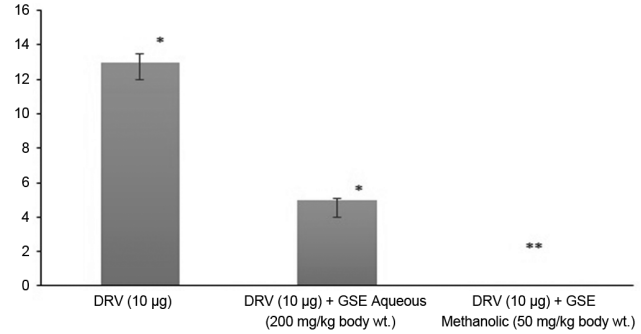

Fig. 5 — Neutralization of *Daboia russelli* venom (DRV) induced hemorrhagic lesion, DRV- *Daboia russelli* venom; GSE- *Gymnema sylvestre* R.Br. leaf extract (GSE). [*P <0.05 between venom control group and aqueous Gymnema +Venom group. **P <0.05 between venom control group and methanolic Gymnema +Venom group]

Discussion

Snake envenomation is a medical disability globally. The World Health Organization (WHO) therefore emphasizes on the testing of anti-snake venom compounds from herbal sources[23]. In India, many plants have been recognised against snake envenomation[24]. The venom present in most snake species exhibits the capacity to induce localized tissue death and bleeding upon intradermal injection[25] (Fig 9). In the present study, *Gymnema sylvestris* R.Br. (GSE) leaf extract could significantly ameliorate viper venom induced defibrinogenating,

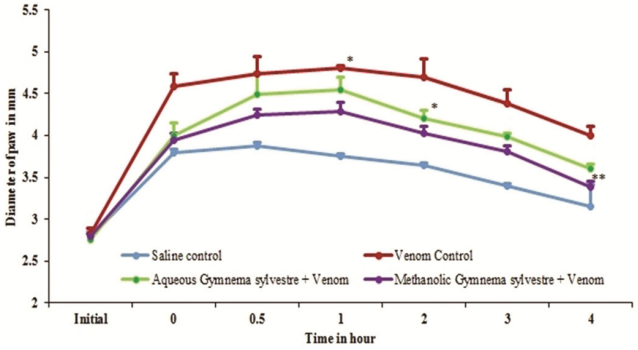

Fig. 7 — Time dependent neutralization of DRV induced edema activity by *Gymnema sylvestre* R.Br. leaf extract. [Results are expressed as mean± SE. *P <0.05 between Group II and Group III. **P <0.05 between Group II and Group IV]

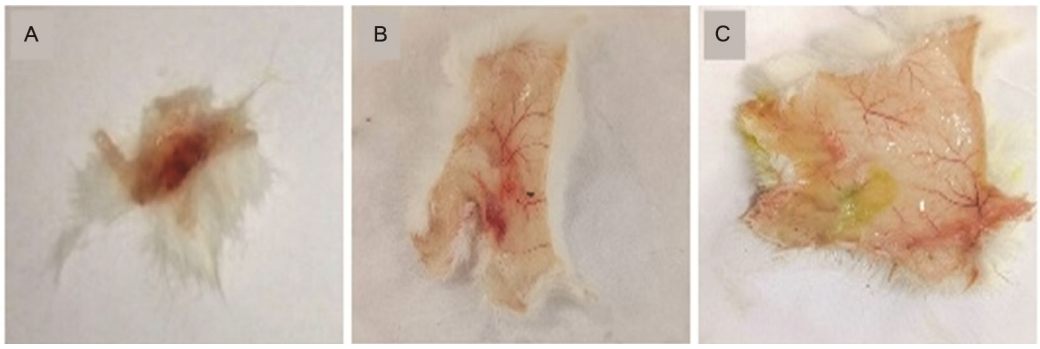

Fig. 6 — Neutralization of Hemorrhagic activity of DRV by *Gymnema sylvestre* R.Br. leaf extract (GSE). Hemorrhagic lesion of mice was induced by (A) DRV; (B & C) DRV along with *Gymnema sylvestre* R.Br. aqueous extract (200 mg/kg body wt.), and methanolic extract. (50 mg/kg body wt.).

Table 3 — Neutralization of edema activity of *Daboia russelli* venom by aqueous and methanolic leaf extract of *Gymnema sylvestre* R. Br

Neutralization of venom	Time (h)	Saline control (DM in mm) (Gr. I)	Venom control (DM in mm) (Gr. II)	Aqueous GSE +venom (DM in mm) (Gr. III)	Methanolic GSE +venom (DM in mm) (Gr. IV)
Edema activity	Before injection	2.80±0.05	2.83±0.05	2.75±0.07	2.80±0.02
	0	3.79±0.04	4.59±0.15	4.00±0.15	3.95±0.08
	0.5	3.88±0.04	4.73±0.2	4.49±0.2	4.24±0.06
	1	3.75±0.02	4.80±0.02 *	4.55±0.14	4.28±0.1
	2	3.64±0.03	4.69±0.2	4.21±0.09 *	4.03±0.07
	3	3.40±0.02	4.38±0.16	3.98±0.04	3.81±0.07
	4	3.15±0.2	4.00±0.1	3.60±0.05	3.39±0.05 **

[DM, diameter; GSE, *Gymnema sylvestre*. Results are expressed as mean± SE;*P <0.05 between Gr. II & III. **P <0.05 between Gr. II & IV]

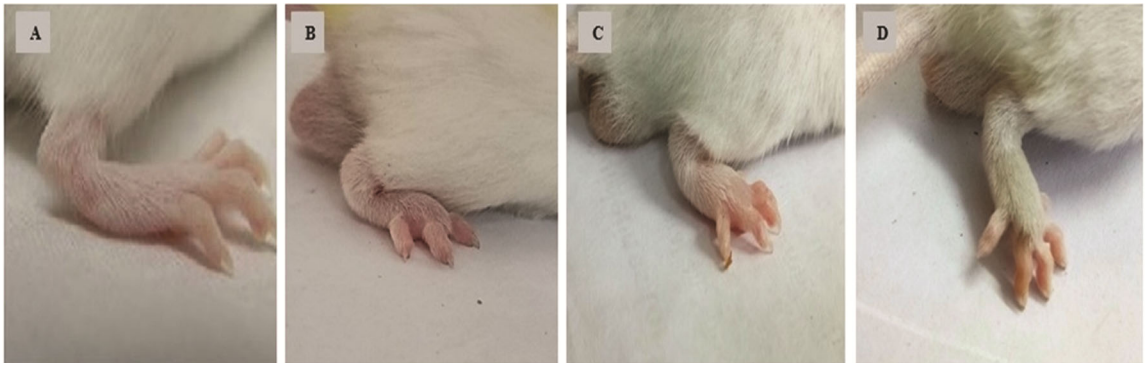

Fig. 8 — Neutralization of Edema activity of DRV by *Gymnema sylvestre* R.Br. extract. (GSE). (A) Saline control**;** (B) *Daboia russelli* venom control (1 µg); (C) DRV (1 µg)+Aqueous *Gymnema* extract (200 mg/kg); and (D) DRV (1 µg)+Methanolic *Gymnema* extract (50 mg/kg)

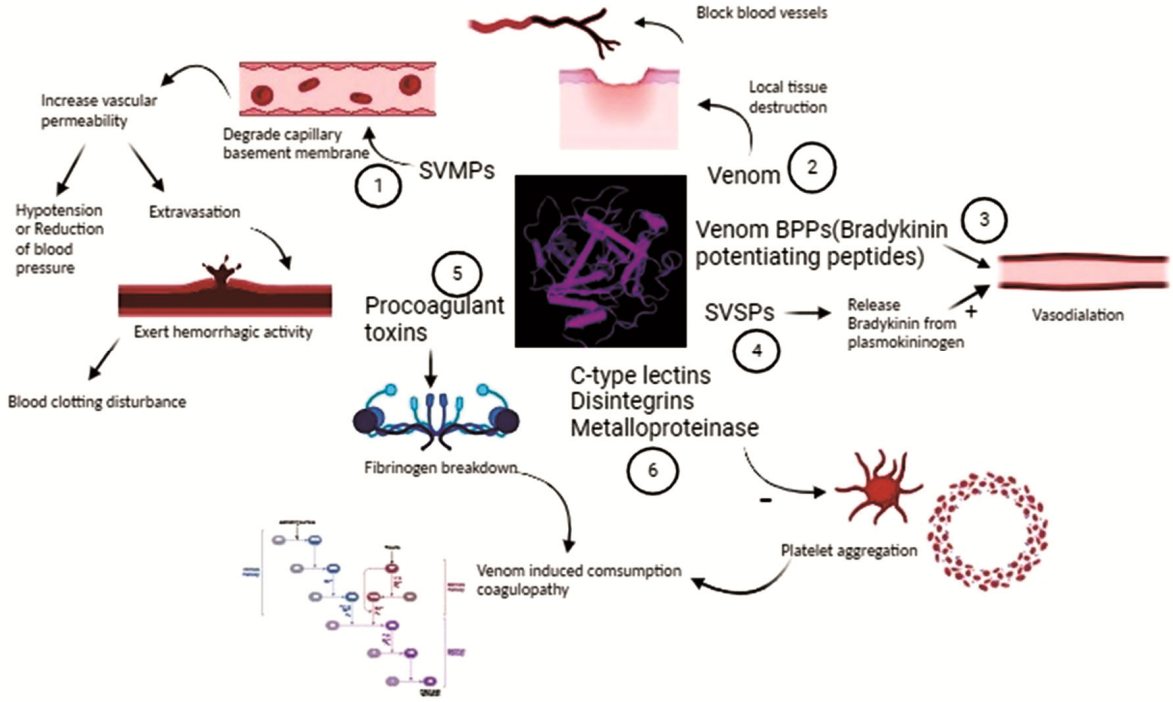

Fig. 9 — Possible mechanism for venom induced changes.

Table 4 — Neutralization of defibrination activity of *Daboia russelli* venom by aqueous and methanolic leaf extract of *Gymnema sylvestre* R.Br.

Neutralization of venom activity	Venom + Aqueous *Gymnema sylvestre* extract @100 mg/mL	Venom + Methanolic *Gymnema sylvestre* extract @20 mg/mL	Fold of protection
Defibrination activity	DRV (1.5 µg)+GSE	DRV (1.5 µg)+GSE	1
	DRV (1.6 µg)+GSE	DRV (1.6 µg)+GSE	1.06
	DRV (1.7 µg)+GSE	DRV (1.7 µg)+GSE	1.13

[DRV, *Daboia russelli* venom; GSE, *Gymnema sylvestre* extract]

Table 5 — Neutralization of PLA_2 activity of *Daboia russelli* venom by aqueous and methanolic leaf extract of *Gymnema sylvestre* R.Br.

Neutralization of venom activity	Venom + Aqueous *Gymnema sylvestre* extract @20 mg/mL	Venom + Methanolic *Gymnema sylvestre* extract @20 mg/mL	Fold of protection
PLA_2 activity	DRV (0.1 µg)+GSE	DRV (0.1 µg)+GSE	1
	DRV (0.2 µg)+GSE	DRV (0.2 µg)+GSE	2
	DRV (0.3 µg)+GSE	DRV (0.3 µg)+GSE	3
	DRV (0.4 µg)+GSE	DRV (0.4 µg)+GSE	NP

[DRV, *Daboia russelli* venom; GSE, *Gymnema sylvestre* extract, NP: Not protected]

haemorrahagic and PLA_2 action. In earlier studies, the anticoagulant, PLA_2 inhibition activity and antihaemorrhagic activities of plant extracts have been noted[26].

Medicinal plants are rich in phytochemicals and have potential as antioxidants. In the present study, *Gymnema sylvestris* R.Br. leaf extract was found to contain flavonoids, quinines, tannins and saponins. *In vitro* phytochemical screening of the leaf extract (both aqueous and methanolic) of *Gymnema sylvestre* R.Br. plant were studied. The tests gave positive result for tannin, saponin, alkaloid, flavonoids, quinone, phenol, glycosides, reducing sugar and protein. Quinone gave negative result for aqueous extract and Phenol gave negative result for methanolic extract. Qualitative phytochemical screening and *in vitro* antioxidant studies were earlier investigated in several plant species including *Gymnema* species[27,28] which is consensus with our present finding.

In DPPH scavenging activity of aqueous and methanolic GSE, the IC_{50} (mg/mL) value was calculated from graph and were found to be 3.24 mg/mL (aqueous) and 2.49 mg/mL (methanolic), respectively. In H_2O_2 scavenging activity of methanolic GSE, the IC_{50} value was calculated from graph and were found to be 6.4 mg/mL (aqueous) and 4 mg/mL (methanolic), respectively which confirms earlier studies[28]. The ferric reducing activity of aqueous and methanolic GSE were found.

It was ascertained that various dosages of *Gymnema sylvestre* R.Br.(200, 500, 800, 1000 and 1500 mg/kg body wt.) did not induce any significant acute toxicity in the mice. This study provides compelling evidence that the administration of both aqueous and methanolic GSE at doses of 400 and 100 mg/kg body wt., respectively confers robust protection against the deleterious consequences of venom at lethal doses. Several such studies on different plants have been earlier reported[29].

The GSE administered at a dosage of 200 mg/kg body wt. for aqueous extract and 50 mg/kg body wt. for methanolic extract exhibited remarkable efficacy in counteracting the haemorrhagic effects induced by *Daboia russelli* venom (DRV). DRV elicits an edema response that is dependent on the dose, characterized by a rapid onset and reaching its pinnacle approximately 30 min after envenomation[30]. When the venom was preincubated with the aqueous and methanolic GSE leaves, a notable reduction in edema was observed. It also neutralizes the venom induced defibrinating activity and gave up to 1.13-fold protection against venom. The leaf extract of *Gymnema sylvestre* R.Br. demonstrated a remarkable ability to effectively neutralize haemorrhage, edema, defibrination and PLA_2 activity induced by *Daboia russelli* venom.

Conclusion

Modern medicine is mostly based on drug discovery whose source is derived from plants. *Gymnema sylvestris* R. Br have been mentioned in *Sushruta*, as a cure for glycosuria and urinary diseases. It is a medicinal herb having multiple potential in *Ayurvedic* and *Homeopathic* systems of medicine. In the present study, we have evaluated the *in vitro* antioxidant and phytochemicals present in this traditional herb. This study corroborates the presence of tannins, saponins, reducing sugars, quinines and flavonoids as phytoconstituents present in the leaf extract. The methanolic extract was found more efficacious in DPPH scavenging, H_2O_2 reducing and ferric reducing activities as evident from its IC_{50} values. Viper envenomation encompasses varied pathophysiological changes which leads to mortality at higher doses. From the acute toxicity studies of plant extract no significant toxicity was found up to 2000 mg/kg body wt. in animal models. In the present work, a significant reduction of venom induced lethality, hemorrhage and edema was evident on treatment with GSE (both aqueous and methanol). This reduction is a manifestation of complexation of polyphenols present in the extract with venom proteins. This is the first report of antivenom potential of this plant .Additional research will in turn unravel and open up avenues for exploring the therapeutic antivenom and antioxidant potential of GSE thereby providing a suitable tool for future management of snakebite.

Ethics approval

The University Animal Ethics Committee, Department of Physiology, Vidyasagar University, Paschim Medinipur, India approved all animal experiments. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Govt. of India (Clearance no. VU/IAEC/CPCSEA/5/9/2024) was followed.

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Conflict of interest

Authors declare no competing interests.

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