Evaluation of Anti-venom Activity of Leaf Extract of *Wedelia trilobata.*

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Abstract:
Wedelia trilobata, commonly known as yellow dot or Singapore daisy and in Hindi as pitabhrunga-raaja and belongs to family Asteraceae. *Wedelia trilobata* used for medicinal benefits in South China, America, Japan, India and other countries for management of many diseases. In folklore, plant is used to treat snake bite, especially in the *Daboia russelli* envenomation. This reseach work was carried out to evaluate the venom neutralization ability of *Wedelia trilobata* against *Daboia russelli* venom. The Ethanolic extract of plant was subjected first time for anti-snake venom activity. Preliminary phytochemical analysis of *Wedelia trilobata* indicated the presence of alkaloids, terpenoids, saponin, tannins, and flavonoids. Administration of the plant extract orally at different dose levels (100, 200 and 400 mg/kg) effectively neutralized *Daboia russelli* venom induced lethality, hemorrhage and necrosis. The above observation confirmed that the plant extract possesses snake venom neutralization capacity.

Keywords: *Wedelia trilobata*, *Daboia russelli*, Hemorrhage, Necrosis.

Introduction:

Snakebites are important public health issue in rural parts of tropical countries. The snakes most commonly associated with human mortality in India are Russell's viper (*Daboia russelli*), saw-scaled viper (*Echis carinatus*), Indian cobra (*Naja naja*), common krait (*Bungarus caeruleus*). India records staggering 10000-15000 deaths annually from snake bite [1]. Antivenom is a biological product used in the treatment of venomous bites or stings. Treatment with antisnake venom (ASV) is effective yet dangerous, because the serum being heterologous and liable to cause sensitivity reactions in patients. Antivenom is immunoglobulin isolated from the serum or plasma

of horses or sheep, immunized against venom of one or more snake venoms. The term "specific" antivenom refers to antivenom that is produced against the venom of a specific snake, and so may be expected to have snake venom specific antibodies, that will neutralize that particular venom [2]. In 2017, World Health Organization (WHO) declared snake bite as "neglected tropical disease", to encourage the research in the field [3]. Herbal medicine is obtained from medicinal plants and are used in the treatment and prevention of diseases. It involves use of folklore treatments using of standardized/titrated herbal extracts. Chemical composition of herbs varies depending on several factors and the action varies from people to people [4]. Herbalism refers to a customary remedial or folklore practice dependent on the use of herbs and herbal extracts. Herbs have advanced their capability to produce active compounds that helps them act on attack from different types of predatory insects, fungi and herbivorous animals. Long before recorded history, plants were in use for treatment of various diseases [5].

Herbal medicines are now in great demand in developing nations for health related issues due to their improved tolerability and compatibility with our system, low possibility of adverse reactions and also inexpensive. Traditional use of herbal medicines for thousand years guides us for the selection, preparation and application of medicinal plants. According to the WHO, the use of herbal remedies throughout the world is two to three times more than that of the conventional drugs. Traditional medicine (including herbal medicines) is defined by the World Health Organization as therapeutic techniques that have existed for centuries before the discovery and imbibition of modern medical system and they are in use until this day. Traditional medicine is the result of generations of indigenous medicine practitioners' therapeutic experience. Traditional medicine preparations include the use medicinal plants, minerals and organic matter etc. Herbal drug comprises of the medicinal plants which are prepared through traditional method [4].

Wedelia trilobata Linn., commonly known as yellow dot or Singapore daisy which is used as traditional herbal medicine, belongs to the family Asteraceae. It has been approved by traditional medical practitioners as an active component in several herbal formulations used for the treatment of liver illnesses, stomach infections, skin infections, fevers, arthritis, and inflammations. *Wedelia* tea is used by women to promote delivery, remove the placenta after birth, or cause abortion [6, 7]. However, the antivenom activity of leaf of *Wedelia trilobata* was not carried out. So present study was undertaken.

Materials and methods:

Collection and identification of leaf

The leaves of *Wedelia trilobata* were collected from medicinal gardens of Institution during the month of July 2020. The plant was authenticated and a specimen was deposited in the institutional herbarium (ref: 19PY012R).

Preparation of extract

The leaf of *Wedelia trilobata* Linn. Were collected and then kept for drying at room temperature, until it was moisture free. The dried leaves are crushed into coarse powder using an electric grinder and subjected to maceration using ethanol as solvent. The extract obtained was concentrated and evaporated under reduced pressure and controlled temperature and stored in desiccators until use [8].

Preliminary qualitative phytochemical investigation

The ethanolic extract of *Wedelia trilobata* was subjected to preliminary qualitative phytochemical examination for identifying the various constituents present in it. The tests were carried out by standard methods [9].

Animals

Albino mice of body weight 18-20 g and rats having weight of 180-200 g was obtained from central animal house, NGSMIPS (Ref. 1781/PO/EReBi/2014/CPCSEA), Paneer, Mangalore. The rats were appropriately grouped and then sheltered in distinct cages. The cages were kept under standard lab conditions of temperature 25 ± 2° with appropriate dark and light cycle of 12 hours. Free access to standard food and water ad libitum was permitted for the animals. The investigation was done in accordance to the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi, India. The Institutional Animal Ethics Committee (IAEC) approval was obtained before initiating the animal studies (NGSMIPS/IAEC/MARCH – 2019/135).

Acute oral toxicity study

Acute toxicity study was conducted to determine the maximum tolerable of the *Wedelia trilobata* ethanolic extract. These study was done in female Albino Rats (150 to 200 g body weight), using "Up and Down Method" as per OECD 425 guidelines. Drug suspension (2000 mg/kg) was orally administered, to the overnight fasted rats. Then the animals were watched continually, once in 30 minutes for the next 4 hrs for any changes in their neurological and general behavioural and finally for death after 24hrs [10].

Dose selection.

For the evaluation of anti-venom ability of the herb, 3 different doses were selected in a way that, the mid dose was $1/10^{th}$ of the highest dose admistered in acute toxicity studies, and a lower dose, i.e. half of the one-tenth dose and one higher dose, which was double of the one-tenth dose (200mg/kg, 100mg/kg, 400mg/kg). The dried crude ethanolic extract of the plant *Wedelia trilobata* was triturated with sodium CMC and the resulting suspension and the aqueous extract was orally administered into the animals. The anti-venom activity of the plant was assessed by using In-vivo and In-vitro model.

LD_{50} evaluation of the venom:

The LD_{50} (median lethal dose; MLD) of *D. russelli* venom was evaluated as per the technique proposed by Theakston and Reid (1983). The lethal dose of venom was determined by inta-peritoneal (i.p) injection of various concentrations of the venom in 0.2ml of PSS (physiological saline) to different clusters (n=6) of albino mice. Mortality was observed after 24h of injection. The LD_{50} is estimated by probit analysis with the confidence limit at 50% of lethality within 24h of the venom injection [11].

In-vivo venom neutralization:

Neutralization of snake venom-induced lethality was studied as described by the method (Theakston and Ried.1983). Six groups of albino mice of either sex, weighing between 18-20g was selected for the study. Each group containing 6 mice. The animals are administered double the MLD of venom intra-peritoneally (i.p), immediately mice were receiving required dose of plant

extracts by oral route. All the animals were observed for mortality up to 24 hours. The number of surviving animals were recorded [11].

Anti-haemorrhagic activity:

The minimum haemorrhagic dose(MHD) of *Daboia russelli* venom was estimated by the procedure proposed by Kondo et al (2). The MHD of venom defined as "the minimum amount of venom that results in a haemorrhagic lesion of 10 mm in diameter 24 hours later when injected intradermally (i.d) into mice/rat" was measured as per previously described method. The neutralization of haemorrhagic action is measured by administering MHD of venom intra-dermally followed by administration of ethanolic extract of leaf at different dose levels p.o. The haemorrhagic lesion was measured after 24hr to determine the leaf extract's anti-haemorrhagic effect on venom [11, 12].

Inhibition of necrotizing activity of venom

Neutralization of venom-induced necrosis was studied according to the mentioned procedure (Theakston. and Ried, 1983). The minimum necrotizing dose (MND) is defined as "the least amount of venom that results in a necrotic lesion of 5 mm diameter 3 days later when injected intra dermally (i.d) into mice/rat" was determined as per previously described method. Inhibition of the necrotizing ability of venom was measured by intradermal (i.d) injection of MND of RVV (Russell's viper venom) followed by oral administration of ethanolic extract of the leaf. The necrotic lesion was estimated after 72h to evaluate the anti-necrotic activity of the leaf extract on venom in vivo [10, 11].

Statistical analysis

The values are stated as Mean ± SEM and Statistical analysis were dose by using ANOVA test followed by Dunnett's test using computer software SPSS version 10. P value less than 0.05 is considered as statistically significant.

Results and discussions

Preparation of extract

A total of 300 grams of the dry coarse leaf powder was subjected to maceration using ethanol. The percentage yield of *Wedelia trilobata* extract was found to be 31.05grms (10.35%).

Preliminary phytochemical analysis of *Wedelia trilobata* leaf extract.

Preliminary phytochemical analysis of *Wedelia trilobata* indicated the presence of alkaloids, terpenoids, saponin, tannins, and flavonoids by using standard chemical tests. Similarly, glycosides, reducing sugras, steroids were found to be absent in the extract.

Acute oral toxicity studies

The ethanolic *Wedelia trilobata* leaf extract was found to be safe at the dose of 2000mg/kg body weight by oral route. The animals were observed for 24 hours for any sign of toxicity following oral administration of the extracts. Animals were found to be well tolerated even after 24 hours. No mortality or signs of toxicity were observed during this study, and the extract was found to be safe at the given dose level. Hence, 3 dose-levels i.e,100mg/kg, 200mg/kg and 400mg/kg body weight were chosen for the current study.

Median lethal dose of the venom (LD_{50}):

The median lethal dose was determined using probit analysis with 50% probability by the analysis (probit analysis) of mortality arising within 24h of the venom injection. The LD_{50} was found to be 20µg/20mg mice (i.p).

In-vitro: neutralization of lethality

The venom inhibition ability of *Wedelia trilobata* leaf extract by in vivo method was measured by i.p injection of 2*LD_{50} of RVV into different group of mice (n=6) followed

by oral administration of various dose of ethanolic leaf extract. The leaf extract at doses 100, 200 mg/kg showed some protection against the venom and 400 mg/kg of plant extract exhibited maximum protection against the lethal activity of $2LD_{50}$ of *Daboia russelli* venom.

Table-1: Effect of ethanolic leaf extract of *Wedelia trilobata* in mice administered with 2*LD_{50} (40µg) of *Daboia russelli* venom (in vivo)

Group	Dose of the plant extract (mg/kg)	Mortality (after 24 hrs) {no of death / no of mice used]	% survival after 24 hrs	Corrected %	Probit
1	Control	6/6	---	4.16	3.25
2	100	3/6	50	50	5.00
3	200	3/6	50	50	5.00
4	400	2/6	66.66	66.66	5.44

Anti-haemorrhagic activity:

The MHD of venom was found to be 40µg. The leaf extract showed significant neutralization of haemorrhage at 200mg/kg and 400mg/kg dose levels.

Table-2: Effect of ethanolic extract of *wedelia trilobata* on the *Daboia russelli* induced haemorrhagic activity in rat.

Dose	Mean dia. Of lesions ± S.E
Control	10.66±0.333
w.t 100mg/kg	10.33±0.211
w.t 200mg/kg	7.00±0.365*
w.t 400mg/kg	3.500±0.224*

The values are expressed Mean ± SEM, n=6 rats in one group. * p< 0.05 significant, when compared with the control group.

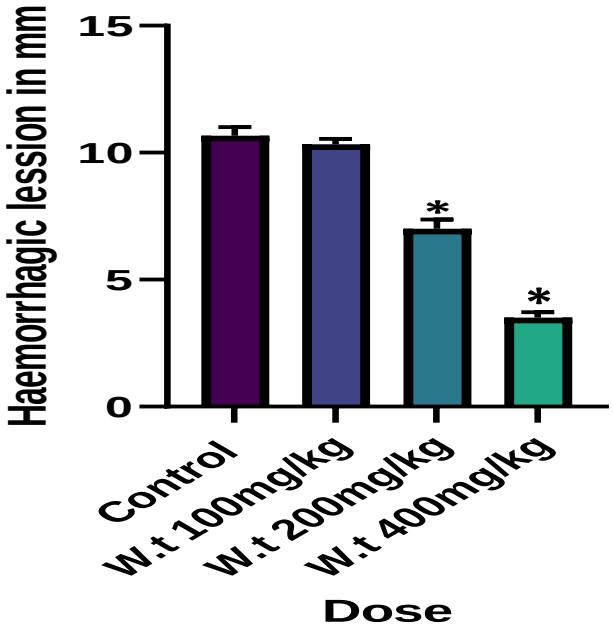

***Figure*-1: Effect of *Wedelia trilobata* extract on venom induced haemorrhage**

Neutralization of necrotizing activity

The MND of the venom was found to be 50µg. The leaf extract showed significant neutralization of necrotic activity of venom at 400mg/kg dose levels.

Table-4: Effect of ethanolic extract of *wedelia trilobata* on the *Daboia russelli* induced necrotizing activity in rat.

Dose	Mean dia. of lesions ± S.E
Control	5.500±0.224
w.t 100mg/kg	5.667±0.211
w.t 200mg/kg	5.000±0.365
w.t 400mg/kg	2.500±0.224*

The data are stated as Mean ± SEM, n=6. * p< 0.05 significant, compared with the control group

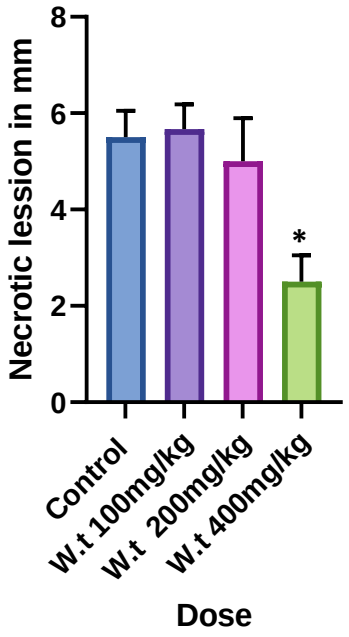

Figure -2: Effect of *Wedelia trilobata* extract on venom induced necrosis

Discussion

The present study was undertaken to perform the preliminary phytochemical evaluation & anti-snake venom activity of plant *Wedelia trilobata* of the family Asteraceae. Preliminary

phytochemical analysis shown the presence of terpenoids, alkaloids, saponin, tannins, and flavonoids.

In traditional system of medicine, various plants were used to treat against snake envenomations [13].

Snake envenomations cause different pathophysiological alterations like as edema, inflammation, haemorrhage, necrosis, changes in blood coagulation system, and ultimately lead to death [1].

DRV is predominantly vasculotoxic and haemotoxic, but it is capable of producing neurotoxic effects as well. Hypotension is the predominant sign in all viperid envenomation and it has been accountable for approximately 38% of mortality in *Daboia russelli* bites [1].

Symptoms of envenomation includes haemorrhage, renal failure, hypotension, local tissue necrosis, edema etc [1]. In current study, comparable order of symptoms was witnessed after the injection of administration of *DRV*.

As per WHO, the compounds having anti-snake venom action should be established for its capability to inhibit the *in-vivo* biological actions of venom such as lethality, haemorhage, necrosis etc. [11].

3 doses of the plant extract 100, 200 and 400 mg/kg body weight, were carefully chosen based on the acute toxicity studies in rats.

Daboia russelli venom has an ability to cause local tissue damages such as necrosis and haemorrhage when injected intradermally. Hence the minimum necrotizing, and minimum haemorrhagic dose estimation proves a reasonable test for assessing the anti-venom activity.

Ethanolic extract of whole plant of *Wedelia trilobata* at dose levels 100, 200 and 400 mg/kg body weight were used for the study.

LD_{50} of the venom was found to be 20µg/20mg mice, MHD was 40µg in rats when injected intra dermally after 24hrs, and MND was 50µg in rats when injected intra dermally after 3 days. Intra vascular haemolysis may contribute acute tubular necrosis and cortical necrosis in victims of *Daboia russelli* bite. Haemorrhagins causes death due to bleeding from vital organs by damaging vascular endothelium. The plant extract at doses 100, 200 mg/kg showed some protection against the venom and 400 mg/kg of plant extract exhibited maximum protection against the lethal activity

of $2LD_{50}$ of *Daboia russelli* venom. The ethanolic extract of *Wedelia trilobata* significantly reduces *Daboia russelli* venom induced necrosis and haemorrhage in rats.

The crunch of ASV availability, particularly in tropical countries reveals a global loss of momentum in anti-venom R&D, improvement and funding. The inability of polyvalent ASV in countering local tissue injury as well forces the scientific world to search for newer substitute ways of handling snake bite cases [14]. Our results confirm that the *Wedelia trilobata* leaf ethanolic extract possesses ability to inhibit DRV. Additional research on separation and identification of active principles from the leaf extract and its anti-snake venom activity could lead to development of new chemical antidote for snake envenomation.

Conclusion

The result obtained from present study shows following conclusions. In the acute oral toxicity studies the extract has been found to be safe up to 2000mg/kg. The ethanolic extract of *Wedelia trilobata* which was administered orally, effectively neutralizes the lethality induced by $2LD_{50}$ of *Daboia russelli* venom in rats. The extract is also able to reduce the venom induced hemorrhage and necrosis.

Our finding confirms the snake venom neutralization capacity of *Wedelia trilobata* ethanolic extract. Hence further study on isolation of active constituent from this plant extract and its anti-snake venom efficacy is required.

Conflict of Interest:

The authors declare no conflict of interest.

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