

Assessment of *In vivo* Antiviral Potential of *Datura metel* Linn. Extracts against Rabies Virus

Soumen Roy, Lalit Samant, Rajeshkumar Ganjhu¹, Sandeepan Mukherjee, Abhay Chowdhary

Department of Virology, Haffkine Institute, Mumbai, ¹Radhika Ayurvedic Research and Development, Pune, Maharashtra, India.

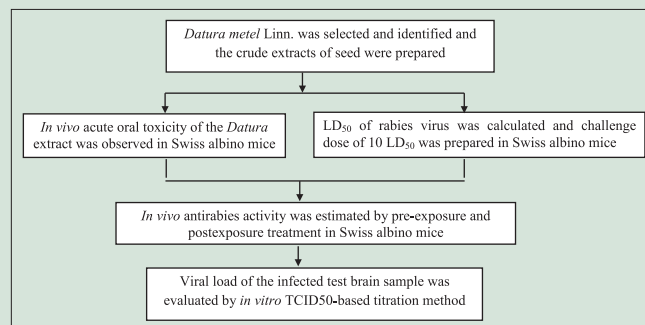
ABSTRACT

Objective: The soxhlet, cold, and ayurvedic extracts of *Datura metel* Linn. were evaluated for *in vivo* antirabies activity. **Materials and Methods:** Soxhlet and cold extraction method were used to extract *Datura* (fruit and seed) extracts, and ayurvedic extraction of *Datura* was prepared. *In vivo* toxicity assay was performed as per the OECD 420. LD₅₀ dose was calculated by Reed and Muench method. The *in vivo* antirabies activity was screened in Swiss albino mice with the virus challenge dose of 10 LD₅₀ (intracerebrally) in both preexposure (PE) and postexposure treatment with oral administration of *Datura* extracts in Swiss albino mice and observed for 21 days. The virus load in the mice brain was evaluated by TCID₅₀ titration method. **Results:** *Datura* (ayurvedic preparation) was found to be nontoxic up to 2000 mg/kg in Swiss albino mice, i.e., 60 mg/30 g of mice, when administered (0.5 ml) orally and observed till 21 days. Up to 20% survival rate on the test group (PE of *Datura* extracts) up to 14 days postinfection as compared to the virus control group (10 LD₅₀) was observed. No survival rate was observed in the postexposure group of *Datura* extract; however, the survival time was increased by 4 days as compared to the virus control group. Viral load of the infected mice brain sample was estimated in vero cell line, and 3 log reduction in the virus titer was observed in test group as compared to the virus control, suggesting that *Datura* extract has an *in vivo* antirabies activity. **Conclusion:** To the best of our knowledge, this is the first study of *in vivo* antiviral activity of an ayurvedic preparation of *D. metel* Linn. against rabies virus. *Datura* extracts have a potential *in vivo* antirabies activity. **Key words:** Antirabies, *Datura*, *in vivo* antiviral assay, rabies

SUMMARY

- In the present study, *Datura metel* Linn. (ayurvedic preparation) extract exhibited survival (20%) in the preexposure (PE) of the virus and the survival

time was increased in the postexposure treatment where the disease was established. The mortality was observed, and the viral load was determined by titration method.



Abbreviation Used: TCID₅₀: tissue culture infectious dose 50; LD₅₀: lethal dose 50; RV CVS: Rabies virus challenge virus standard; PE: Pre exposure; IC: intracerebral; PI: post infection; FITC: Fluorescein isothiocyanate

Correspondence:

Dr. Soumen Roy,
Department of Virology,
Haffkine Institute for
Training, Research and Testing, A D Marg,
Parel, Mumbai - 400 012,
Maharashtra, India.
E-mail: soumenroy118@gmail.com
DOI: 10.4103/pr.pr_121_17

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INTRODUCTION

Rabies is a fatal viral zoonotic disease which causes encephalitis in all warm-blooded animals, including humans, continued to be a serious problem in Africa.^[1] Rabies, a disease known to humanity for over 100 years, is till date considered a fatal killer once the symptoms begin to appear. The burden of the disease is quite high with nearly 55,000 people dying of this dreadful disease globally, of which approximately 29,000 deaths being reported annually from India alone. Rabies caused by a single-stranded, negative-sense RNA virus is maintained in nature by a variety of animal reservoirs and primarily infects the central nervous system (CNS), resulting in progressive encephalopathy and ultimately death in an infected human.^[2] Rabies prevention can be achieved by elimination of exposure and by vaccination through PE prophylaxis and postexposure treatment.^[3] PE rabies prophylaxis affords a measure of protection for unrecognized rabies exposures and simplifies postexposure treatment. Postexposure treatment is recommended following exposure to a potentially rabid animal and involves treatment of wound and administration of rabies vaccine as well as rabies immunoglobulin for individuals not previously vaccinated. Despite the availability of prevention and prophylaxis measures, rabies continues to be a threat globally.

Rabies is an acute viral infection of the CNS that is usually fatal in humans and animals. Rabies causes about 55,000 human deaths annually worldwide, with 95% of human deaths due to rabies occur in Asia and Africa. India has the highest rate of human rabies in the world, primarily because of stray dogs, whose number has greatly increased since a 2001 law forbade the killing of dogs. A total of 20,000 people are estimated to die every year from rabies in India more than one-third of the global toll.^[4,5]

Plants serve as a source to treat different ailments.^[6] Many useful drugs have been developed from medicinal plants used in traditional medicine in the treatment of a variety of illnesses.^[7]

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Cite this article as: Roy S, Samant L, Ganjhu R, Mukherjee S, Chowdhary A. Assessment of *In vivo* Antiviral Potential of *Datura metel* Linn. Extracts against Rabies Virus. *Phcog Res* 2018;10:109-12.

Datura has long been used as an extremely effective treatment for asthma symptoms. The active antiasthmatic agent is atropine, which causes paralysis of the pulmonary branches of the lungs, eliminating the spasms that cause the asthma attacks. The leaves are generally smoked either in a cigarette or a pipe. This practice of smoking *Datura* to relieve asthma has its origins in traditional ayurvedic medicine in India. *Datura* also has a wide range of traditional application including epilepsy, hysteria, insanity, heart, and skin diseases. It also cures mental diseases and relieves pain.^[8]

Therapeutic human rabies immunoglobulin and vaccine are available against rabies virus, but it is ineffective once the virus enters the CNS. There was only one reported case of a female survival from rabies virus by inducing coma and treating her with a combination of known antiviral drugs (Milwaukee protocol).^[9] Therefore, the search for more effective antiviral agents against viral infections of the CNS is a necessary and highly desirable task. This necessitates the need for the development of novel antivirals against rabies virus. Plant secondary metabolites have tremendous potential to be antiviral agents.

The potential of *Datura* plant extract was exhibited by *in vitro* model where *Datura* extract was found to have a pronounced *in vitro* antirabies activity.^[10]

However, the *in vivo* antiviral potential of *Datura* was not explored earlier. This study intended to evaluate the *in vivo* antirabies activity of *Datura* extract in a murine model.

MATERIALS AND METHODS

Collection of plant material

Datura fruit was collected from the Phool market, Dadar, Mumbai. Plant specimen was authenticated and deposited in Blatter Herbarium, Department of Botany, St. Xavier's College, Mumbai, Maharashtra, India.

Preparation of plant extracts

The *Datura* seed separated from the fruit and was air dried and then was soaked in cow urine for 12 h. After 12 h, the seed was separated from the cow urine and air-dried. The dried seed was then boiled for 3 h in cow milk. After treatment with milk, the *Datura* seed was separated from the milk, and the seed cover was peeled out from the *Datura* seed. The peeled *Datura* seed was air-dried and then fine grinded and stored for further use. During processing, the seed powder was dissolved in water and was used for the antiviral assay.^[11]

Animal study

Swiss albino mice aged from 4 to 6 weeks were purchased from Haffkine Institute (animal house), and the study was approved by the Institutional Animal Ethics Committee (IAEC) HITRT/IAEC/19/2013. The challenged virus standard strain of fixed rabies virus (RV CVS) was passaged in Swiss albino mice (30 μ l intracerebrally) to increase the virus titer, and RV CVS stock suspension (10% w/v) was stored at -80°C . The LD₅₀ dose was calculated by Reed and Muench method, and based on the 1 LD₅₀ of the RV CVS, a challenge dose of 10 LD₅₀ RV CVS was used in PE and postexposure treatments with *Datura* extracts.^[12]

In vivo toxicity of *Datura* extracts

The oral toxicity study was performed using fixed dose method (OECD guideline no. 420). The mice (3 weeks old) were provided from the Animal House Department, Haffkine Institute for the acute oral toxicity of the *Datura* extracts.

A single dose of *Datura metel* extracts was prepared as per 2000 mg/kg body weight of the Swiss albino mice for the fixed dose method. Swiss albino mice were grouped as per the sex and weight of the animal, and six mice per test group were considered for the toxicity study.

The concentration of 2000 mg/kg each extract was administered orally in the test group and observed for 21 days for mortality and morbidity. After 21 days, the animals were sacrificed and the toxicity was observed in major viral organs by for histopathological examination.^[12]

Titration of stock virus

The challenge virus (RV CVS) was stored as a 10% suspension in -80°C in the ampule/cryovials. Shortly before the test is carried out, a vial is taken from the stock and thawed rapidly under the tap. Serial tenfold dilution was then prepared (10^{-1} up to 10^{-7}) in the vials and the LD₅₀ was calculated.^[13]

Preexposure treatment

In the PE treatment, the test group of Swiss albino mice (10 mice/group) was administered orally (20 mg/ml) with the single dose of *Datura* extracts for 3 days, followed by the challenge dose of (30 μ l intracerebrally) 10 LD RV CVS was inoculated onto the test (PE) Swiss albino mice. The virus control group of mice (10 mice/group) was inoculated intracerebrally with 10 LD₅₀ RV CVS. Postinfection (PI) after 6 h, the test groups were orally administered (20 mg/ml) *Datura* extract for 14 days. All the group of mice was observed mortality up to 21 days.^[14]

Postexposure treatment

In the postexposure treatment, the test group of Swiss albino mice (10 mice/group) was inoculated intracerebrally (30 μ l) with the challenge dose of 10 LD₅₀ RV CVS followed by the oral administration (after 6 h) of the *Datura* extracts (20 mg/ml) in the test group of mice. The virus control group (10 mice/group) of mice was inoculated intracerebrally with 10 LD₅₀ RV CVS and the extract control group (10 mice/group) of mice was administered with the *Datura* extracts. The test group and the extract control group of mice (10 mice each) were administered up to 14 days and all the groups of mice were observed mortality up to 21 days.^[14]

Virus-releasing study by titration

The infected brain suspension was serially diluted in medium and infected in a 96 well plate with vero cell line and incubated for 72 h in a 37°C , CO₂ incubator for virus titration. After incubation, the infected cells were fixed with chilled acetone and stained with FITC-tagged antibodies and were observed under a fluorescent inverted microscope. The viral load was calculated by TCID50 titration method and the virus titer was determined by fluorescent antibody test method.^[10]

Statistical analysis

Statistical analysis was performed using two-way analysis of variance test with GraphPad Prism 5.1 software Inc, (La Jolla California USA).

RESULTS AND DISCUSSION

In vivo toxicity of crude extracts

Datura extract was not found to be toxic up to 2000 mg/kg body weight of Swiss albino mice, i.e., 60 mg/30 g of mice when administered (0.5 ml) orally and observed till 21 days. The LD₅₀ titer was calculated by the Reed and Muench method. The titer of $10^{4.75}$ LD₅₀/0.03 ml RV CVS (1 LD₅₀) was obtained. Based on the 1 LD₅₀, the challenge dose of 10 LD₅₀ RV CVS was used for the *in vivo* antirabies activity by PE and postexposure treatment.

In a PE treatment, we observed 20% survival rate on the test group (PE of *Datura* extracts) even after 21 days PI as compared to the virus control group of mice where mortality was observed on 10 day PI [Figure 1].

In a postexposure treatment, no survival rate was observed in the test group; however, survival time was increased by 4 days in the test group of mice as compared to the virus control group of mice where the mortality was observed in 10 days PI [Figure 2].

In vivo titration of the infected brain for rabies virus

In vivo, antirabies activity of *Datura* extracts was evaluated in Swiss albino mice when challenged with 10 LD₅₀ RV CVS by PE and postexposure treatment. The infected test brains along with the virus control brains were harvested and titrated in vero cell line to determine the presence of the virus and the virus titer as compared with the virus control.

We observed 3 log and 1 log decreased in the virus titer of the test group of *Datura* PE treated and *Datura* postexposure treated infected brain suspension, respectively, as compared to the virus control brain suspension. The *Datura* (PE and postexposure) treatment significantly reduced the viral load in the Swiss albino mice when challenged with the 10 LD₅₀ RV CVS as compared with the virus control [Figure 3].

A single dose of *Datura* extracts (20 mg/ml) was administered orally followed by a challenge dose of 10 LD₅₀ RV CVS (30 µl) inoculated intracerebrally in (3 weeks old) Swiss albino mice for both PE and postexposure trail experiment. In the PE treatment of *Datura* extract, we observed 20% survival rate on the test group up to 14 days PI as compared to the virus control group of mice where mortality was observed on 10 days PI. However, in the postexposure treatment, no survival rate was observed as the test group mice died as compared to the virus control group. However, survival time was increased by 4 days in the test group of mice as compared to the virus control group of mice where the mortality was observed in 10 days PI.

In the similar study, it was found that when the mice were administered with 1000 mg/kg dose of hydroethanolic extract *Phytolacca dodecandra* leaves, 33.3% survival rate was observed when challenged with rabies virus as compared to the virus control. However, 10% survival rate was found at the 600 mg/kg dose of the extract in mice and no survival was at 300 mg/kg dose of hydroethanolic extract *P. dodecandra* leaves when challenged with rabies virus as compared to the virus control.^[15]

Datura is a wild plant having various medicinal and pharmacological properties. Phytochemical of the plant are alkaloids, atropine, scopolamine, tannin, saponins, glycosides, phenol, sterols, lignins, fats, carbohydrates, and proteins.^[16]

Datura plant contains high levels of tropane alkaloids such as atropine, hyoscyamine, and scopolamine. Atropine and scopolamine are muscarinic antagonists which can be used to treat Parkinson's diseases and parasympathetic stimulation of the eye, heart, urinary, respiratory, and gastrointestinal tract. Anticholinergics are a class of compounds that inhibit parasympathetic nerve impulses by selectively blocking the binding of the neurotransmitter acetylcholine to its receptor in nerve cells. Atropine is an anticholinergic agent that blocks the neurotransmitter acetylcholine in the central and the peripheral nervous system. Rabies virus also interacts with the acetylcholine receptor in the CNS.^[17]

In the study, we observed efficacy in the PE treatment with *Datura* extract but in the postexposure treatment where the infection is established in the CNS, only the survival time was increased but no protection was observed. The possible mechanism might be the masking the acetylcholine receptors and preventing the entry and thereby neutralizing the virus. Earlier studies suggest that *D. metel* extracts have a wide range of antibacterial and antifungal activity.^[18]

In vitro study of *Datura* extract exhibited antirabies activity.^[10] We observed that in *in vivo* study of *Datura* exhibited promising antirabies activity. The *Datura* extract prolonged the survival time by 4 days in the postexposure treatment whereas 20% survival rate was observed in the

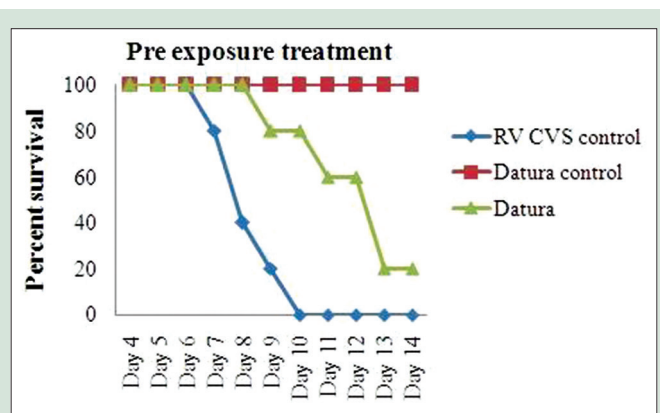


Figure 1: Pre-exposure treatment of *Datura* (ayurvedic) extracts against a challenge dose of 10 LD₅₀ challenged virus standard strain of fixed rabies virus. The results are expressed as standard error of the mean for each Group

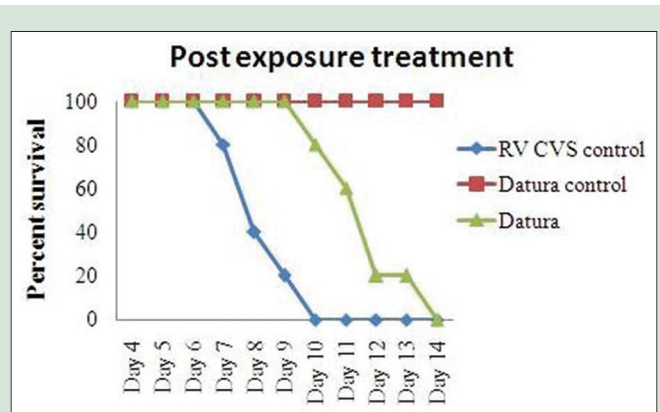


Figure 2: Postexposure treatment of *Datura* (ayurvedic) extracts against a challenge dose of 10 LD₅₀ challenged virus standard strain of fixed rabies virus. The results are expressed as standard error of the mean for each Group

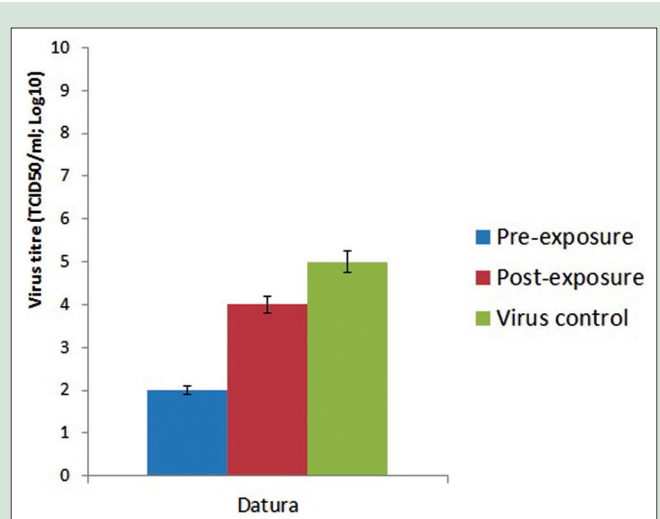


Figure 3: Titration of the test and virus control infected brain suspension. The results are expressed as standard error of the mean for each Group

PE treatment in a murine model. Further research needs to be done on the mechanism of *Datura* to assess its protective role in rabies treatment.

CONCLUSION

Datura extract has an *in vivo* antirabies activity, as 20% survival rate was observed in the PE treatment. Moreover, also the survival time was increased in the PE treatment after the disease was established in a murine model. Further research needs to be done on the mechanism of *Datura* to assess its protective role in rabies treatment.

Acknowledgment

We would like to thank Haffkine Institute, Mumbai, for providing animals and NIMHANS, Bengaluru, for providing RV CVS for the research work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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