Evaluation of Phytolacca dodecandra stem and leaf hydroethanolic extract's in vivo anti-rabies activity

Arava Vidyadhari [1*†], Usa Suvarna [2], Shaik Ismail [2], Pantapalli Lokeh [2], Sonagiri Naveen [2], Susrla Saichandra [2], Thota Renuka [2], Srikanta Saikamakshi [2]

[1] *Department of Pharmacology, Saastra College of Pharmaceutical Education & Research, Near Varigonda Jwalamukhi Temple, Muthukur Raod, Kakupalli, Nellore-524 311 Andhra Pradesh, India, Tel.: +91 6302192037*

[2] *Saastra College of Pharmaceutical Education & Research, Near Varigonda Jwalamukhi Temple, Muthukur Raod, Kakupalli, Nellore-524 311 Andhra Pradesh, India*

Abstract

A mouse model was utilized in the study to assess the antirabies effectiveness of a hydroethanolic extract of the stems and leaves of Phytolacca dodecandra (L' Herit) (Phytolaccaceae), one of the plants that is frequently used in Ethiopia for the traditional treatment of rabies in humans and animals. Based on the difference in the survival rate and duration (days) of the group of mice challenged with the rabies virus, the antirabies activity of both portions of the plant extract in doses of 300, 600, and 1000 mg/kg was compared with the negative control (CVS-11). The results revealed that mice's survival times were considerably reduced ($P<0.05$) when compared to both the positive and negative control groups, but not significantly ($P>0.05$) when compared to all doses of the plant's stems and 300 and 600 mg/kg dosages of its leaves. However, when compared to the mice's respective negative control group, the mice's survival duration increased significantly ($P<0.05$) after receiving a dose of 1000 mg/kg of the plant extract from the leaves. The discovery suggested the presence of some anti-rabies activity in P. dodecandra leaf extract at higher doses, but more research is required to clarify its active components.

Keywords: Anti-rabies, In vivo, Hydroethanolic Extract, Phytolacca dodecandra

*Corresponding author.

†E-mail: aravavidhyadhari@gmail.com

1 INTRODUCTION

All warm-blooded animals, including humans, can develop encephalitis from the lethal viral zoonotic disease rabies [1]. Even though the disease first showed up in the fourth century B.C., a clear diagnosis could not be made until the first century B.C. Since Louis Pasteur created the first rabies vaccine for humans in 1885, there have been tremendous advancements in this sector, including improvements in laboratory diagnosis, immunization, and rabies management in wild, domestic, and farm animals.

An estimated 55,000 people are killed by rabies every year, predominantly in Asia and Africa. Although rabies-related deaths are a substantial cause of death in many underdeveloped nations, the economic impact of rabies is likely much bigger than overt human mortality. The projected global economic cost of rabies is more than $583 million, which does not account for the suffering that rabies-related deaths cause in families and communities. Due to the many treatments available for the illness, costs related to rabid animals and those incurred from the moment an animal was suspected of being rabid were substantial. Even though rabies can be effectively managed in domesticated animals by a variety of practical and generally accessible vaccines, canine rabies remains a severe issue in Africa, particularly Ethiopia.

Herbal remedies made of herbs, herbal materials, herbal preparations, and completed herbal products (including plant parts or other plant materials as active components) are included in traditional medicine (TM). 70% to 95% of the population of most developing nations, particularly those in Asia, Africa, Latin America, and the Middle East, relies on traditional medicines to cure various disorders. To maintain health and treat illnesses in both humans and animals, herbal remedies can be made from plant roots, leaves, bark, seeds, berries, or flowers. The mixture of secondary products found in plants that have historically been employed as sources of medicine and are still used as the foundation for many pharmaceuticals today is usually what causes plant materials to have good therapeutic effects [2].

90% of the population in Ethiopia relies on TMs to treat illnesses in both humans and animals, as they have done since the beginning of time. Ethiopia's populace, both urban and rural, uses traditional medicine widely. This widespread use may be due to traditional medicine's cultural acceptance, physical accessibility, and financial affordability when compared to modern medicine. Different indigenous groups and regions of Ethiopia have reported using a variety of traditional anti-rabies herbs for the treatment of rabies in both humans and animals. Phytolacca dodecandra is one of the known plants that is frequently used in Ethiopia for the conventional treatment of rabies in both humans and animals. However, some research has been done to determine whether these and other unnamed ethnomedicinal plants are effective at treating

rabies [3].

Even though many different vaccines can be used to cure rabies, it is still important to keep looking for novel substances that include anti-rabies agents. Nearly 25% of contemporary medications come from plants that were first utilized traditionally [4]. In many regions of Ethiopia, people use traditional herbal medicine as one of their primary forms of treatment for rabies. Despite this, traditional anti-rabies herbal medicines in Ethiopia are not as well supported by research or understood as traditional anti-rabies vaccines. Ethiopian Health Research Institute (EHNRI) and other health centres/institutions in Ethiopia reported that adverse fatal side effects and cases of rabies deaths after traditional treatment were the biggest problems due to the non-standardization of constituents, quality, and efficacy of these traditionally used anti-rabies herbal remedies, despite the lack of published evidence on the overall problems posed by these remedies. However, locals and traditional rabies healers in Ethiopia assert that these indigenous plants may treat rabies in both humans and animals [5]. Furthermore, the exploration of a novel anti-rabies component generated from plants is crucial for the advancement of biomedical research as well as the fight against rabies in humans and animals.

Different researchers have noted the use of about twelve traditional antirabies plants by various indigenous people from various regions of Ethiopia to treat rabies in both humans and animals. Only Deressa and his colleagues [6] evaluated the effectiveness of anti-rabies activities of crude extract of Salix subserrata and Silene macroselen plants in mice, which increased the survival period (Days) of experimental mice compared to the control group of mice. Modern pharmaceutical practices, however, have not been used to evaluate the efficacy of these plants against rabies. Among the many ethnic groups of Ethiopia, P. dodecandra has a long history of usage in the treatment of rabies in both humans and animals. However, no research has been done to assess the anti-rabies activity of extracts from P. dodecandra's stems and leaves in both in vivo and in vitro systems. Therefore, the study's goals were to compare the hydroethanolic extract of P. dodecandra's stems and leaves to a negative control group of Swiss albino mice to assess its anti-rabies effects [7].

2 MATERIALS AND METHODS

Preparation and Extraction of Plant material

P. dodecandra stems and leaves, which are utilized by traditional healers, were harvested from their native environment, cleaned carefully, chopped into pieces, air-dried (dried inside without exposure to sunlight), reduced to little bits, and stored in a deep freezer until the

plant material was extracted. Plant materials were weighed using a precise digital balance before being macerated in 80% ethanol and concentrated following the instructions provided by Debella [8]. In a one-litre Erlenmeyer flask, 100 g of powdered plant material was steeped in 1000 ml of 80% ethanol. The flask containing the mixture of dissolved plant components and 80% ethanol was sealed with cotton wool and shaken continuously for 24 hours at 120–190 rpm. After 24 hours, the supernatant was concentrated using a rotary evaporator and filtered using Whatman (No. 1) filter paper. The remaining solvent was then evaporated to dryness using a vacuum and a water bath at +40°C. The residue was given extra solvent, and it was filtered twice more. Finally, during the duration of the investigation, the yield of extracts was kept at -4°C in an airtight container [4].

Animals Used in Experiments and Their Care

The male and female Swiss albino mice used in the experiment ranged in age from 4 to 7 weeks and weight from 20 to 35 g. The Ethiopian Health and Nutrition Research Institute's regular laboratory animal house served as the breeding facility for all laboratory animals used in this experiment (EHNRI). All of the animals used in the experiment were treated following the accepted practices for the use and care of laboratory animals, and the experiment was approved by EHNRI. Animals were kept in a littered, clean metal cage with a 12-hour light/dark cycle, with the litter being changed every three days, after being taken from the laboratory animal unit [9]. To evaluate the anti-rabies activity of both portions of the plant extract, mice were randomly divided into six groups and housed in a single cage with other mice of the same sex. The animals were given unlimited access to fresh water and pelleted food (Mice cubes). Before beginning any experimental technique, the animals were allowed to acclimatize under controlled settings for at least three days, and each animal was utilized only once. All experimental animals received a consistent food regimen. According to WHO guidelines for anti-rabies pre-exposure prophylaxis, the investigator and all staff caring for animals that had been infected with the rabies virus received three doses of the commercially available pre-exposure anti-rabies vaccine Verorab (PVRV, Sanofi Pasteur, France) intramuscularly on days 0, 7, and 21 [10].

Virus strain and its Inoculation

The virus was diluted with phosphate-buffered saline (PBS) solution in Atlanta, Georgia, USA to contain 50–200 mouse intra-masseter 50% lethal doses ($MIMLD_{50}$) per 0.03 ml for a single challenge, as determined by Reed and Muench methods before this experiment was carried out. The virus was created from suckling mouse brains infected with rabies. The protocols for this study adhered to the regulations for good laboratory practice (GLP) in rabies as well as the recommendations for the care and welfare of research animals [11]. Based on the method

Wunderli and his colleagues used, CVS-11 viral inoculation via intramasseter muscle was carried out. Consequently, all mouse groups received a day 0 inoculation of the CVS-11 virus strain. After an hour of a CVS challenge, treatment groups of mice received doses of 300, 600, and 1000 mg extracts of both the stems and leaves of P. dodecandra for seven consecutive days.

TABLE 1

Experimental mice have been divided into various groups, each with a description

Groups	No. of mice in the group	Description
AS	12	Received 300 mg/kg of P.dodecandra stems extract for 7 days, Per Os
BS	12	Received 600 mg/kg of P.dodecandra stems extract for 7 days, Per Os
CS	12	Received 1000 mg/kg of P.dodecandra stems extract for 7 days, Per Os
AL	12	Received 300 mg/kg of P.dodecandra leaves extract for 7 days, Per Os
BL	12	Received 600 mg/kg of P.dodecandra leaves extract for 7 days, Per Os
CL	12	Received 1000 mg/kg of P.dodecandra leaves extract for 7 days, Per Os
D	12	Placebo received 1 ml of Distilled water (dH2O), Per Os

Experimental Design

The treatment group and the harmful control group were given random assignments to the animals [Table 1]. Three dose levels (300, 600, and 1000 mg/kg) for each part of the plant extract were used in the treatment groups, which were divided into three sub-groups for each dose of the two parts of the plant extract. Instead of both portions of the plant extracts,

TABLE 2

Effects of P. dodecandra stems and leaves extracts on mice exposed to the rabies virus in terms of percentage survival and mean survival time

Groups	Survival n (%)	Death n (%)	Days of survival (Mean±SD)
AS (300 mg/kg)	0 (0%)	10 (98.86%)	9.87± 1.68
BS (600 mg/kg)	1 (9.7%)	8 (86%)	12.41±5.38
CS (1000 mg/kg)	0 (0%)	10 (98.32%)	10.38± 1.65
AL (300 mg/kg)	0 (0%)	11 (99%)	10.82± 4.82
BL (600 mg/kg)	1 (9.3%)	10 (90.9%)	8.07± 0.86
CL (1000 mg/kg)	5 (38.92%)	7(66.4 %)	22.86± 5.42
D (Placebo)	0 (0%)	12 (100%)	9.18± 1.46

TABLE 3

Comparison of treatment group mice's survival times and survival rates with the mice in the negative control group

Groups	Survival time/Life expectancy Mean Difference (days)	†P-value	‡P-value
AS (300 mg/kg)	-0.86	0.241	nd
BS (600 mg/kg)	-3.19	0.124	0.432
CS (1000 mg/kg)	-1.29	0.059	nd
AL (300 mg/kg)	-1.75	0.368	0.435
BL (600 mg/kg)	-0.93	0.123	nd
CL (1000 mg/kg)	-12.98	0.000	0.187

†P-value for the Student's t-test comparison of the survival time with the negative control group (D); P-value for the Chi-square using Fisher's exact test comparing the survival rate with the negative control group (D); AS, BS &CS (300, 600 & 1000 mg/kg stems, respectively); AL, BL & CL (300, 600& 1000 mg/kg leaves, respectively), nd=not done

one placebo-negative control group of mice was given dH_2O and then challenged with CVS-11. Table 1 lists categories of experimental animal species. Utilizing a metal cage and a 12-hour light/dark cycle, administering the extracts along with distilled water (dH_2O) was carried out using a litter and an intragastric needle based on the animal's body weight in a 1-ml vehicle [12].

Determination of Mortality Rate

The clinical indications of rabies and/or a direct fluorescent antibody test were used to estimate mortality rates (FAT). All mice were kept and monitored for a total of 30 days after the virus challenge. After contracting the rabies virus, they were checked daily for symptoms of the disease, such as rough fur, tremors, incoordination, paralysis, and prostration. Any symptoms of the disease were noted daily on the mouse history cards [13]. Direct FAT was stained using rabies anti-nucleocapsid antibodies that are available for purchase (monoclonal antibodies) and are labelled with fluorescein isothiocyanate (FITC)-dye ® (rabies conjugate anti-nucleocapsid, BIORAD, South Africa). The working dilution was made following the manufacturer's instructions [14].

Opening the mice's skulls and collecting their brains following the Dean and Albelseth-specified technique allowed for the direct FAT confirmation diagnosis of rabies. In a nutshell, the tails of mice and the rostral end of the heads were pined in a vice that was installed on the operating table. On the dorsal surface of the head, a midline incision was created with a scalpel and blade. The brain tissues were then revealed by scissor-cutting the top of the skull (Calvarium), which was done after removing the skin, aponeurosis, and temporal muscles and reflecting them laterally. The cerebellum, hippocampus, brain stem, and any other brain tissues that were present were included in the brain sample, and an impression smear was created for direct FAT. According to Kissling [15] specified steps, a standardized methodology for the direct FAT was carried out. In a nutshell, brain tissues were processed as impression smears on slides, which were then air-dried for 15–20 minutes at room temperature. Acetone was used to fix the smears for an hour overnight at -20°C.

Brain impression smears were stained for 30 min. at 37^0 C. with FITC-labelled anti-rabies conjugate. Finally, after too much conjugate had been removed from the slides and mounting medium had been applied, the slides were examined with a fluorescence microscope using a 40X objective to look for the distinctive green fluorescence linked to the rabies antigen [16]. All rabies-infected tissues that were processed on infected mice were disposed of as medical waste, and all samples used for rabies diagnosis were handled with the proper biosafety precautions to prevent direct contact with possibly contaminated tissues or fluids.

Statistical Analysis

To estimate the survival rate and mean survival time (Days) in each group of mice, data were analysed using SPSS version 20 and various statistical techniques. To compare the number of survivors (Survival rate) in various groups of mice, The significance of observed variations in the mean survival time (Days) of the mouse groups was determined using the Student's t-test. In all two-tailed statistical studies, P values less than 0.05 were regarded as significant [17].

3 RESULTS

The study's findings included % survival and mean survival time for groups of mice infected and treated with both parts of plant extract as well as infected but treated with both parts of plant extract. No mice were protected from rabies deaths from groups of mice treated with hydroethanolic extract of stems of P. dodecandra at doses of 300 mg/kg and 1000 mg/kg, and only 1 (9.7%) of mice were protected when the same plant part administered at dose level of 600 mg/kg. These groups of mice were infected with the rabies virus but not treated with any plant extracts, showing a 0% survival rate and 9.18 days survival time. The average number of days that mice were exposed to P. dodecandra stem extract at doses of 300, 600, and 1000 mg/kg survived was 9.87, 12.41, and 10.38, respectively (Table 2). In terms of their percentage survival and mean survival duration, groups of mice treated with all doses of a plant extract from the roots and the negative control group of mice showed almost similar findings. In groups of mice given hydroethanolic extracts of P. dodecandra leaves at dosages of 300, 600, and 1000 mg/kg, 0 (0%), 1(9.7%), and 5 (38.92%) mice, respectively, were protected from rabies fatalities.

The mice in the 300, 600, and 1000 mg/kg leaf extract of the P. dodecandra group had mean survival times of 10.81, 8.18, and 22.83 days, respectively [Table 2]. In contrast to the effects of all dosages of the plant's stem extract, a relatively greater percentage survival (33.3%) and mean survival length (22.83 days) was achieved when an extract of P. dodecandra leaves was provided at a dose of 1000 mg/kg. The findings showed that none of the doses of either portion of the plant extract substantially ($P>0.05$) increased the survival rate of mice compared to the mice in the negative control group. The outcome also revealed that none of the concentrations of plant extract taken from the stems, nor the 300 mg/kg and 600 mg/kg taken from the leaves, substantially lengthened the time that mice survived (days) when compared to the mice in the negative control group ($P>0.05$). However, the mice's survival time (days) was considerably extended by the plant extract from the leaves at a level of 1000 mg/kg ($P<0.05$) when compared to the negative control groups [Table 3].

4 Discussion

Comparing the negative control group to neither of the plant extract's parts significantly increased the percentage of mice in the treatment group that survived. The lack of chemicals (if present, they exist in trace amounts) that impede the growth and pathogenesis of the rabies virus in examined mice may be the cause of the ineffectiveness of all dosages of the plant's stems and 300 and 600 mg/kg doses of its leaves. On the other hand, the purpose of anti-rabies vaccinations is to increase the production of anti-virus antibodies that limit infection from mice that have received an injection of the rabies virus (CVS-11). The oral treatment of rabies-infected mice with a higher dose (1000 mg/kg) of P. dodecandra leaves given over seven days increased the mean survival time significantly ($P<0.05$) compared to the negative control group, indicating that the hydroethanolic extract of P. dodecandra leaves has some antirabies activity. The effect of P. dodecandra leaf extract on survival time was dose-dependent (the higher dose showed higher survival time than lower doses), whereas the effect of stems extract did not depend on dose (none of the three doses of stems extract significantly extended survival time or prevented death in mice).

The presence of the ribosomal inhibitory protein (RIP) dodecandrin, which has been shown to have antiviral properties, may be a sign that the hydroethanolic extract of P. dodecandra leaves has some anti-rabies action. The impact of P. dodecandra leaf extract on tested mice in extending survival time may be attributable to various secondary metabolites of the plant parts or their combined actions. Higher doses of the plant part's leaf extract may contain chemicals that have an impact on the rabies virus's in vivo pathogeneses and reproduction than lesser doses do. The leaf extract of P. dodecandra has been shown in other earlier research to have moderate effectiveness against the coxsackie virus in an in vitro system. The epizootic lymphangitis-causing bacteria Histoplasma capsulatum var. farciminosum, Staphylococcus aureus, and Pseudomonas aeruginosa were all significantly inhibited by hydroalcoholic extracts of P. dodecandra's aerial portions, according to Tadeg and his colleagues.

There were further reports of plants with antiviral and/or anti-rabies action when evaluated in vitro or in vivo systems. Alamanda schottii leaves and flowers were evaluated by Muller and his colleagues using a technique that tested the extract's in vitro antirabies activity. Aqueous extract of Nepeta nepetella leaves also exhibits antiviral activity, according to Abad and his coworkers. Contrarily, Deressa and his coworkers reported that crude hydromethanolic and chloroform extracts of S. macroselen roots and chloroform and aqueous extracts of S. subserrata leaves significantly increased the survival period of experimental mice when compared to the mice in the negative control group.

5 Conclusion

When compared to negative control groups, the hydroethanolic extract of P. dodecandra's stems and leaves did not significantly increase the survival rate of mice. However, when compared to a negative control group of mice, a leaf extract from the plant had some anti-rabies action at a treatment level of 1000 mg/kg. The results showed that an extract of P. dodecandra leaves had some anti-rabies action at higher dose levels. The active components of the P. dodecandra leaf extract that has anti-rabies activity should thus be further investigated to identify other beneficial chemicals.

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Nil.

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