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Green Synthesis of Silver Nanoparticle Using *Catharanthus roseus* Extract For Pharmacological Activity

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ABSTRACT

Nano biotechnology gives emphasis for the synthesis of nanoparticles using living organisms such as microorganisms, plant extracts or plant biomass in an eco-friendly way. Among the various agents used for nanoparticle synthesis, plants have found the important application. The present study was designed to screen the neuroprotective effect of *Catharanthus roseus* (Linn.) on streptozotocin induced diabetic neuropathy in rats. Diabetes was induced in rats with a single intraperitoneal injection of streptozotocin (55 mg/kg b.w). The ethanol extract of *Catharanthus roseus* at a dose of 100, 200 and 400 mg/kg of body weight was administered at the single dose per day to diabetes-induced rats for a period of 12 weeks. Neuropathic pain was assessed in diabetic rats with various painful procedures viz., hot and cold-water tail immersion test performed to assess the degree of thermal, mechanical, cold hyperalgesia and locomotor activity as well as motor coordination. The biomolecules found in plants induce the reduction of Ag+ ions from silver nitrate to silver nanoparticles (AgNPs). The aqueous leaves extract of Catharanthus roseus was used as reducing and stabilizing agent for the synthesis of the silver nanoparticle. The synthesized nanoparticle is confirmed by the change of color from transparent yellow to dark brown indicates the formation of silver nanoparticles. FTIR spectra were used to monitor the quantitative formation of silver nanoparticles. The plant-based route could be considered an environmentally friendly, safe and economic biological method for the silver nanoparticles production.

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INTRODUCTION

In the present scenario, nanotechnology is an important enabling active area of research in modern material sciences. Nanoparticles deals with the synthesis and control of matter in scales less than 1µm, normally from 1 to 100 nanometers (nm) [1]. Nanoparticles show completely new or improved properties and have the wide scope for their diversified application based on specific characteristics such as size, distribution, and morphology. Silver nanoparticles have found various and important applications for their bactericidal and fungicidal activity[2]. Antimicrobial effect is due to blockage of respiratory enzyme pathways, alterations of microbial DNA and the cell wall[3]. Historically, the synthesis of metallic nanoparticles utilized chemical reducing agents such as hydrazine, sodium citrate, and sodium borohydride to create uniform suspensions[4]. However, the chemical method is harmful in some way as the chemicals used are toxic, flammable, low synthesis rate etc. In the current phase, green synthesis of nanoparticles is exploited to improve and to protect the environment by the use of chemicals. Raveendran et al. 2003 suggested three important factors, which should be considered for the synthesis of nanoparticles, solvent choice, the use of reducing agent and the use of non-toxic material for nanoparticle stablisation[5]. Recently, biological entities serving as both reducing and stabilizing agents for green synthesis of metallic nanoparticles[6]. Utilizing biological organisms such as microorganisms[7], enzymes[8] and plant extract or plant biomass could be an excellent alternative to chemical and physical methods for the production of nanoparticles in a cheap and eco-friendly manner compared to physical and chemical methods.

Synthesis of nanoparticles using plants can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell culture[9]. The microbial enzymes and secondary metabolites with anti-oxidant or reducing properties are usually reducing metal compounds into their respective nanoparticles. Plants have been reported to be used for the synthesis of metal nanoparticles of gold and silver and of a gold-silver-copper alloy [10-14]. Colloidal silver is of particular interest because of its distinctive properties such as good conductivity, chemical stability, and catalytic and antibacterial activity[15-16]. In during present study, we found that plant extracts prepared from *Catharanthus roses* can be used for the synthesis of silver NPs under bright conditions. The objective of the present study was the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver

nitrate by the aqueous extract of medicinal plants and evaluation of synthesized silver nanoparticles against animal toxicity.

MATERIAL AND METHODS

Experimental

Silver nitrate was purchased from Merck Chemicals. All glassware sterilized with nitric acid, further with distilled water, and dried in the oven before use. *Catharanthus roseus* leaves were collected from the college campus in the month of March.

Preparation of leaf extract

The fresh leaves were washed several times with running tap water and after that with distilled water. Around 20 g of leaves were weighed and boiled for 1h in 100 mL double distilled water at 60°C and then the extracts were filtered through Whatman filter paper. Then the filtered extract was stored in the refrigerator at 4^0C for further use in the synthesis of silver nanoparticles.

One pot green synthesis of silver nanoparticles

100 mL (1mM) aqueous solution of silver nitrate was prepared in the volumetric flask. Then 1.0, 2.0, 3.0, 4.0 and 5.0 mL of leaf extract were added separately to 10mL aqueous silver nitrate solution kept in separate beakers at room temperature. The solution was kept in dark chamber until solution color changes to yellow to dark yellow. After, 15 min, the solution turns yellow to yellow-red or dark brown indicating the formation of silver nanoparticles.

Sample	Plant extract (ml)	$AgNO_3$ solution (ml)
A	10	-
1A	1	10
2A	2	10
3A	3	10
4A	4	10
5A	5	S10

Structural studies

FTIR has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules, which is used to search the chemical

composition of the surface of the silver nanoparticles and identify the biomolecules for capping and efficient stabilization of the metal nanoparticles. There were many functional groups present which may have been responsible for the bio-reduction of Ag+ ions. The band intensities in different regions of the spectrum for plant extract and silver nanoparticles were analyzed and are shown in Figure.

Acute Toxicity Studies

All the animals were fed with rodent pellet diet and water was allowed ad-libitum under strict hygienic condition. The animals were fasted overnight prior to the experiment. Fixed-dose method as per OECD Guideline No. 425 method, given by CPCSEA was adopted for toxicity studies. The study was conducted by prior permission of institutional animal ethical committee (IAEC registration no. 731/Po/Re/2002/CPCSEA, approval no. CBPC/IAEC/2016-17/11). The Wister rats were divided in to control and test group each containing 6 animals. The test groups of rats were administrated with the dose of 25, 200, 500 &2000 mg/kg of extracts. Carefully observe all the rats and any sign of toxicity in the first four hours, after the administration of extracts and daily following that for the period of 14 days.

Animal Activity

Selection of animals, caring, and handling

The Wistar rats (Wistar strain 150-200 g) of either sex were used. After randomization into various groups, animals were accustomed for a period of 10 days under standard husbandry condition.

Room temperature: 23 ± 3°C

Relative humidity: 50 ± 20%

12 hrs dark and light cycle.

Acute toxicity study

Method

Acute toxicity tests are generally the first tests conducted. They provide data on the relative toxicity likely to arise from a single drug exposure. The study was conducted after obtaining

Institutional animal ethical committee clearance according to Rule 170, Department of Ayush, Government of India and OECD guidelines 420

Material: 1) Wistar albino rat

2) *Catharanthus roseus* Linn

3) Gum acacia

4) Distilled water

Procedure

Rats were fasted for 24 hrs prior to drug administration. Six animals were used. MSB uniformly dispersed in 2% Gum acacia suspension was administered as a single oral dose equivalent to 2000 mg/kg body weight. Food was withheld for a further 4 hrs. animals were observed individually at least once during the first 30 min after dosing, and then periodically during the first 24 hrs (with special attention during first 4 hrs), and daily thereafter for a period of 14 days. Mortality, if any, was determined over a period of 2 weeks (OECD, 2001). LD_{50} was calculated as per OECD guidelines.

Acute toxicity study of *Catharanthus roseus* on animal model

Plant materials: The leaves of *Catharanthus roseus* was collected from a college campus in the month of March.

Extract preparation: The leaves of *Catharanthus roseus* were collected and dried under shade and ground into powder. Aqueous extract of *Catharanthus roseus* leaves was done in the Department of Pharmacology,

Acute toxicity study: Acute toxicity study of aqueous extract of the leaves of *Catharanthus roseus* was determined in Wistar albino rats (150-180 gm) according to the OECD guidelines No.420. Based on performed toxicity tests the LD50 dose was selected in three doses of 100, 200, 400 mg/kg P.O.

Drugs and Chemicals: *Streptozotocin* was obtained from Sisco research laboratories Pvt. Ltd, Mumbai, India, and gabapentin was purchased from Swapnaroop drugs &

pharmaceuticals, Aurangabad, Maharastra, India. All other chemicals and reagents used were of analytical grade.

Animals used: Adult healthy Albino rats of Wistar strain of either sex weighing between 180-250 gm was gathered from the Central animal house, Aurangabad. The rats were housed in polypropylene cages under standard laboratory conditions 23±2^{0}C with 12hr light dark cycle and had free accesses to water with standard chow diet. Animal care should be taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Approval was taken from the Institutional Animal Ethics Committee for the study.

Experimental design: **Experimental design:**

In the present investigation, 36 rats were taken and divided into six groups of 6 rats in each. Out of 6 groups, five were made diabetic with a single dose of the prepared solution of *Streptozotocin* 55 mg/kg body weight in cold citrate buffer (PH 4.5, 0.01 M) was administered intraperitoneally. After 72 hrs blood, glucose level of surviving rats was measured and rats with fasting blood glucose levels above 250 mg/dl were used for further study.

The study of test compound and standard drugs were dissolved in distilled water and administered orally with the help of the gastric oral tube. Rats were divided into the following groups;

Group-1: Normal control rats (Distilled water 5 ml/kg, p.o)

Group-2: Diabetic control rats (*STZ* 55 mg/kg, i.p)

Group-3: Diabetic rats served with *gabapentin* (10 mg/kg, p.o)

Group-4: Diabetic rats served with *Catharanthus roseus* extract (100 mg/kg, p.o)

Group-5: Diabetic rats served with *Catharanthus roseus* extract (200 mg/kg, p.o)

Group-6: Diabetic rats served with *Catharanthus roseus* extract (400 mg/kg, p.o)

The study period was carried out for 3 weeks, behavioral parameters and fasting blood glucose levels as well as changes in the body weights of the animals were determined on 0, 1^{st},2^{nd} and3^{rd} week respectively.

Evaluation of Behavioral activity:

Assessment of hyperalgesia and Allodynia:

Cold-water tail immersion test: In cold-water tail immersion test, distal 5 cm of the tail was immersed in a cold-water container by maintaining a constant temperature (10°C). Duration of time taken for withdrawal of tail from cold water was noted. A cut-off time of 20 sec was maintained to prevent tissue injury. The procedure was repeated three times for each animal and the mean values are taken into consideration. The decrease in tail contact time with cold water was pointing towards nociception, whereas prolonged contact time was noted as the anti-allodynic effect.

Hot water tail immersion test: In hot water tail immersion test, heat hyperalgesia was measured by immersion of terminal part of the tail (1 cm) in warm water (52.5 ± 0.5°C). The duration of tail withdrawal reflex was recorded, as a response to heat thermal sensation and a cut-off time of 15 seconds was maintained. Shortening of tail withdrawal time is an indication for thermal hyperalgesia.

Synthesis of silver nanoparticles:

Silver nitrate ($AgNO_3$) was obtained from Sigma Aldrich and 1 mM $AgNO_3$ solution was prepared and stored in the amber color bottle and used in future experimental work. 10 ml plant leaf broth was added to 90 ml 1 mM aqueous silver nitrate with constant stirring and allowed to react at ambient conditions for reduction into Ag^+ ions. The observed color change of reaction mixture from transparent yellow to dark brown indicates the formation of silver nanoparticles from leaves. The content was centrifuged at 20,000 rpm for 20 minutes. The supernatant obtained was used for the analysis of acute toxicity study. Further, the reduction of the Ag+ ions was monitored over time by FTIR spectral analysis.

RESULTS AND DISCUSSION

Synthesis and FTIR Spectra Analysis: The green synthesis of silver nanoparticles through herbal extracts was carried using aqueous silver nitrate solution. Medicinal plant including

Catharanthus roseus was used for the synthesis of a silver nanoparticle. The method utilizes a non-toxic, agent, which functions as both reducing and stabilizing agent during synthesis. The mechanism of the reaction involves the reduction of the aqueous metal ion with plant leaves extract. Plant extracts color changes after the completion of the reaction. In addition, it is well known that silver

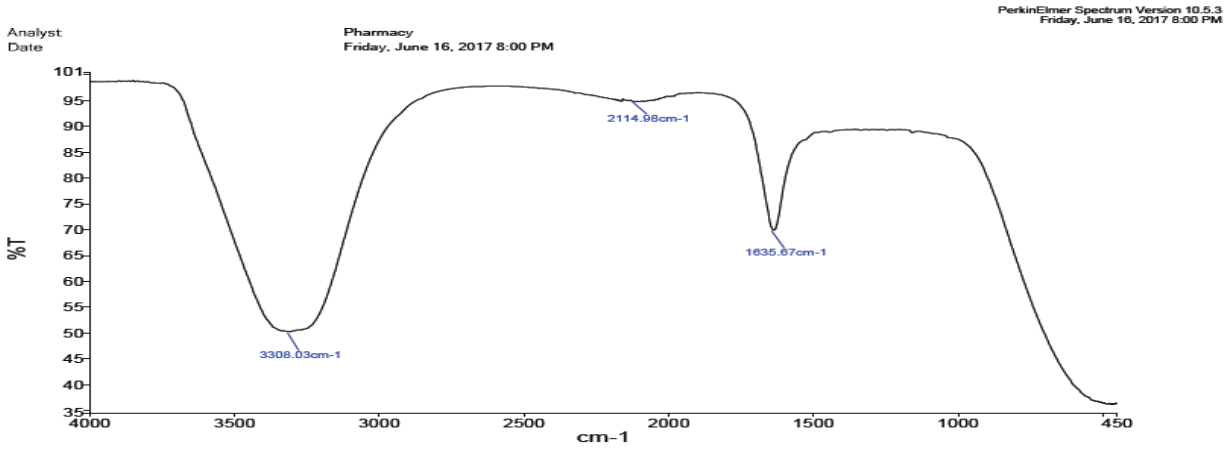

Fig: 1 FTIR spectra of Catharanthus extract

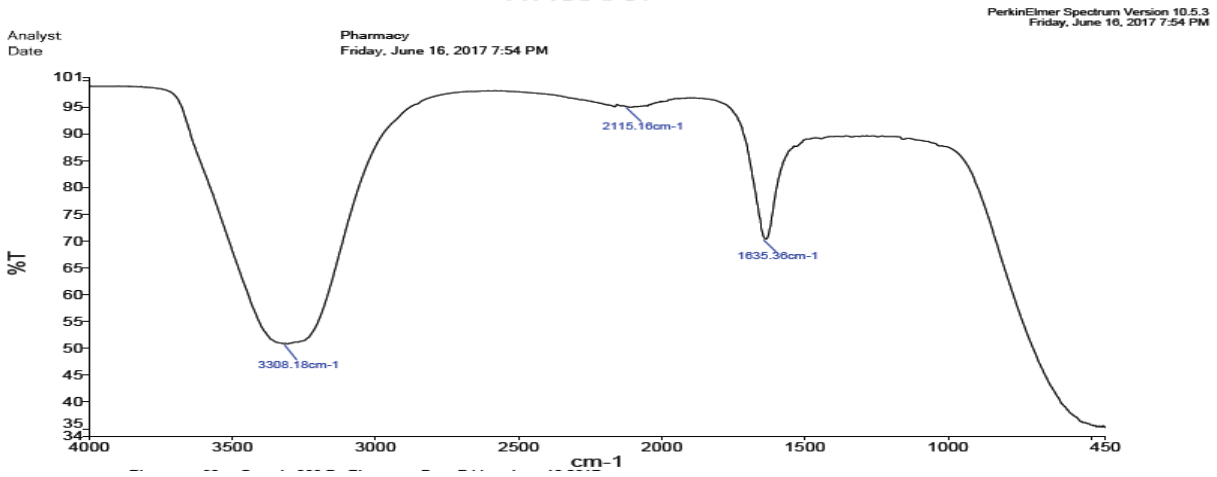

Fig: 2 FTIR spectra of synthesized silver nanoparticle

Fig. 3 Synthesis of silver nanoparticle indicated by change in color

Acute toxicity study

Wister rat (1)	Wister rat (2)	Wister rat (3)	Wister rat (4)	Wister rat (5)	Wister rat (6)
100 mg/kg	200mg/kg	500mg/kg	1000mg/kg	1500mg/kg	2000 mg/kg
Mortality not observe	Mortality not observe	Mortality not observe	Mortality not observe	Mortality not observe	Mortality not observe

***In -vivo study* (Acute toxicity studies)**

Acute toxicity studies and dose determination The LD_{50} of *Catharanthus roseus* as per OECD guideline falls under class four with no signs of acute toxicity with up to a maximum dose of 2400 mg/kg. Any changes in normal behavioral pattern or signs and symptoms of toxicity and mortality were not observed up to this dose level.

Effect of *Catharanthus roseus* on behavioral parameters:

Hot and cold-water tail immersion test:

Streptozotocin (STZ) induced hyperglycemia results in progressive heat hyperalgesia and cold allodynia, reflected as the shortening of tail withdrawal latency in comparison with normal rats (group 1). Leaves extract treated rats were started to show early significant improvement in tail withdrawal latency at dose 400 mg/kg ($P<0.001$) from the 4^{th} week and remaining

doses 100, 200 mg/kg were showed the effect on 8th week for hot water tail immersion test (Table-1). In cold-water tail immersion, all three doses of *Catharanthus roseus* 50, 100 and 200 mg/kg were significantly improved tail withdrawal latency in a dose-dependent manner. Whereas, *gabapentin* 10 mg/kg significantly ($P < 0.05$) improved the tail withdrawal latency when compared with group 2 rats (Table-2).

Table-1: Effect of leaves extract on rats subjected to hot water tail immersion test

Groups	Reaction time (Sec.)			
	Week 0	4th Week	8th Week	12th Week
Control	9.67±1.211	9.83±0.753	10.33±0.516	10.17±0.753
STZ control	5.67±1.366	4.83±1.169	4.67±1.211	5.17±0.753
Gabapentin(10 mg/kg)	5.33±1.211	8.50±1.049***	11.50±1.049***	13.67±1.211***
Leaves Ext. 50mg/kg	6.00±0.894	6.67±0.516	7.50±0.548**	8.10±0.753***
Leaves Ext. 100mg/kg	5.17±1.169	6.33±0.816	7.67±0.816***	8.83±0.753***

All values are presented as Mean±SD, (n = 6), p<0.05*, p<0.01**, p<0.001*** when compared to disease group.

Table-2: Effect of leaves extract on rats subjected to cold water tail immersion test

Sr. No	Groups	Reaction time (Sec.)			
		Week 0	4th Week	8th Week	12th Week
1	Control	10.83±0.753	11.33±0.516	11.17±0.753	11.50±0.548
2	*STZ* control	6.50±0.548	5.33±0.516	4.83±0.753	4.33±0.516
3	*Gabapentin*(10mg/kg)	6.17±0.983	9.50±1.049***	12.67±0.816***	14.33±0.816***
4	Leaves Ext. 50mg/kg	6.17±0.753	7.00±0.894*	7.83±1.169***	8.00±0.632***
5	Leaves Ext. 100mg/kg	6.33±0.816	7.33±0.516**	8.17±0.753***	9.33±0.516***

All values are presented as Mean±SD, (n = 6), p<0.05*, p<0.01**, p<0.001*** when compared to disease group.

CONCLUSION

This work indicates that important herbal extract could be used as an efficient and potential green material for the reliable synthesis of silver nanoparticles. The synthesized phyto nanoparticles have exhibited a wide range of activities to the bacteria strains and reveal high

efficacy of silver Nanoparticle as a strong antibacterial agent. Thus, this phyto nanoparticles has the potential for the development of drugs for various diseases and useful in biomedical application.

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