ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research

Available Online at www.jbpr.in

CODEN: - JBPRAU (Source: - American Chemical Society)

Index Copernicus Value: 72.80

PubMed (National Library of Medicine): ID: (101671502)

Volume 7, Issue 1: January-February: 2018, 68-74

Research Article

# EVALUATION OF ANTI-HYPERTENSIVE ACTIVITY OF NORDOSTACHYS JATAMANSION AGAINST CADMIUM CHLORIDE INDUCES HYPERTENSION IN ALBINO RATS

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Received 02 Dec. 2017; Accepted 20 Jan. 2018

**ABSTRACT**

*Nardostachysjatamansi* is a very effective, potential and safe drug for the management of patients with essential hypertension along with dietary restrictions and modified lifestyle. The sesquiterpenevaleranone isolated from the subterranian parts of *Nardostachysjatamansi* (DC) is found to having sedative, tranquilizing and antihypertensive properties**.** *Nordostachysjatamansi*whole plant was collected from herbal garden of National Institute of Ayurveda (NIA), Jaipur. Genuine sample of plant material was weighed by the electronic monopan balance and a thin layer of sample was spreaded on a white colour sheet. By bull lens, the layer was examined for foreign matter. Moisture content was determined by placing weighed sample of 5gm of Plant material in oven at 105ºC for 5 hours, and calculated weight of sample for every 30 minute. The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per liter Freshly prepared extracts were tested for the presence of various active phytocompounds like phenols, tannin, Flavonoids, protein, reducing sugar, carbohydrates, lipids, Saponin, triterpenoid alkaloid, resins, volatile oils, anthraquinone and Quinone. Identification can be effected by observation of spots of identical RF value. Acute Toxicity Study *(*according to OECD 423 guideline). B. Anti-hypertensive activity ($CdCl_2$ Induced hypertension)

**Keywords:** *Nardostachysjatamansi,* Acute Toxicity, hypertensive activity, Ayurveda

## 1. INTRODUCTION:

Hypertension is a serious disease affecting a significant population globally The reported prevalence of hypertension varied around the world, with the lowest prevalence in rural India (3.4% in men and 6.8% in women) and the highest prevalence in Poland (68.9% in men and 72.5% in women). Awareness of hypertension was reported for 46% of the studies and varied from 25.2% in Korea to 75% in Barbados; treatment varied from 10.7% in Mexico to 66% inBarbados and control (blood pressure < 140/90 mmHg while on antihypertensive medication) varied from 5.4% in Korea to 58% in Barbados.

### 1.1 Plant collection

*Nordostachysjatamansi*whole plant were collected from herbal garden of National Institute of Ayurveda (NIA), Jaipur and authenticated from CSIR-national institute of science communication and information resources, New Delhi.

**Table 1: Table showing chemicals used during the dissertation work**

| | | |
|---|---|---|
| Hydrochloric acid | Sodium hydroxide | Toluene |
| Distilled Water | Acetic acid | Hypophosporus |
| Ethanol | Potassium chromate | Lead acetate |
| Petroleum ether | Potassium iodide | Potassium bismuth iodide |
| Potassium Ferro cyanide | Ammonia | Vanillin |

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| | | |
|---|---|---|
| Sulphuric acid | Ammonium chloride | Ferric chloride |
| Potassium thiocynate | Sodium sulphide | Chloroform |
| Per chloric acid | Sodium potassium tartrate | Pyridine |
| Barium chloride | Iodine solution | Magnesium turning |
| Methanol | Copper sulphate | Petroleum ether |
| Molisch`s reagent | Picric acid | Glycerine |

### 1.2. Equipment's and Apparatus

List of equipment's and apparatus used during the dissertation work.

**Table 2: Table showing list of equipment's & apparatus.**

| | |
|---|---|
| Digital balance | Condenser |
| Magnifying Bull Lens | Heating mantle |
| Hot air oven | Hot plate |
| Silica crucible | Water bath |
| Grinder | Common glass wares |
| Muffle furnace | Rotary shaker |
| Desiccators | |

### 1.3. Physiochemical Properties

**a. Determination of foreign matter:** Genuine sample of plant material was weighed by the electronic monopan balance and a thin layer of sample was spreaded on a white colour sheet. By bull lens, the layer was examined for foreign matter

**b. Determination of moisture content**: Moisture content was determined by placing weighed sample of 5gm of Plant material in oven at 105ºC for 5 hours, and calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight would be recorded.

**c. Determination of p: H**Practically it means the quantitative indication of the acidity or basic nature of a solution. The pH of a given solution is measured by using digital pH meter.

**d. Determination of Total Ash:** Silica Crucible was cleaned, dried well & labeled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample was put in the Silica crucible. The drug was spread evenly into a thin layer.

**e. Determination of Water Soluble Ash:** Water soluble ash value was determined as per Pharmacopoeia of India 1996. Boil the total ash for 5 minutes with 25 ml of water; collect the insoluble matter in a Gooch's.

**f. Determination of Extractive values:** Extraction of plant material is done as per API suggested method

**g. Determination of Alcohol Soluble Extractive:** It was taken Macerate 5 gm of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allow to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 1050º C , to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

**h. Determination of Water Soluble Extractive:** Proceed as directed for the determination of alcohol-soluble extractive, using distilled water instead of ethanol.

**i. Determination of Petroleum Ether Soluble Extractive (Fixed Oil Content):** Transferred a suitably weighed quantity (depending on the fixed oil content) of the air-dried, crushed drug to an extraction thimble, extract with solvent ether (or petroleum ether, b.p. 40º C to 60ºC) in a continuous extraction apparatus for 6 hours.

Filtered the extract quantitatively into a tarred evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105º C to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

**1.4. Phytochemical Properties:** Freshly prepared extracts were tested for the presence of various active phytocompounds like phenols, tannin, Flavonoids, protein, reducing sugar, carbohydrates, lipids, Saponin, triterpenoid alkaloid, resins, volatile oils, anthraquinone and Quinone.

**a. Chromatography plates-**

T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Mercks) were used. (Each plate dimension is 10 cm long and 2 cm width)

**b. Activation of pre-coated Silica gel G60F254** Dry in hot oven at $105^0$ C for one to two hour.

**c. Preparation of Test sample:** Alcoholic Extract

**d. Preparation of mobile solution:** Toluene: Ethyl acetate9.3:0.7. (v/v)

**e. Sample application:** Sample was applied with the help of capillary 1(one) cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate.

**f. Visualization:** AnashaldehydeSulphuric Acid

**g. Rf. Value:** Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

**Table 3: Physiochemical Properties**

| Sr. No | Tests | Observation |
|---|---|---|
| 1 | Foreign matter | 4.27% |
| 2 | Moisture content | 9.34% |
| 3 | pH | 5.6 |
| 4 | Total Ash | 7.35% |
| 5 | Water Soluble Ash | 5.97% |
| 6 | Acid Insoluble Ash | 2.47% |
| 7 | Alcohol Soluble Extractive | 9.24% |
| 8 | Water Soluble Extractive | 17.31% |
| 9 | Petroleum Ether Soluble Extractive | 6.34% |

**Table 4: Phytochemical Properties**

| S. No | Name of Test | Observation |
|---|---|---|
| 1 | Tests for Carbohydrates- | |
| A | Molisch's Test | +ve |
| B | Benedict's test | -ve |
| C | Barfoed's test | -ve |
| D | Fehling solution test | +ve |
| 2 | Tests for Alkaloids- | |
| A | Mayer's reagent test | +ve |
| B | Dragondroff's reagent test | -ve |
| C | Wagner's test | +ve |
| D | Hager's Test | -ve |
| 3 | Test for Amino acids- | |
| A | Ninhydrin test | +ve |
| 4 | Protein | |
| A | Xanthoprotic test | +ve |

| | | |
|---|---|---|
| B | Millons test | -ve |
| C | Biuret test | +ve |
| 5 | Tests for Glycosides- | |
| a | Borntragor's Test | |
| 6 | Test for Phenolic Compound: | +ve |
| 7 | Test for Saponin(Foam test)- | +ve |
| 8 | Test for Steriods( Salkowaskireaction) | +ve |
| 9 | Test for Tennins – | |
| A | $Fecl_3$ | -ve |
| B | Lead acetate | +ve |
| c | Pot. Dichromate | -ve |

**TLC:**

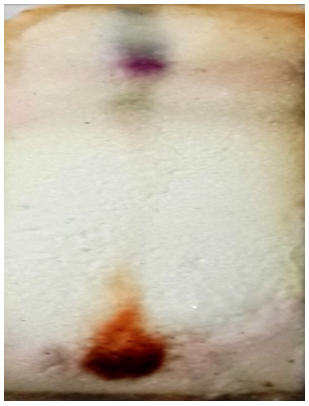

**Fig. 1: TLCPlate of *Nardostachysjatamansi***

$R_f$ Value= 0.73, 0.82, 0.86, 0.97

**1.5 PHARMACOLOGICAL STUDY**

**1.5.1 Pre-Clinical Study:**

A. Acute Toxicity Study *(*according to OECD 423 guideline).
B. Anti-hypertensive activity ($CdCl_2$ Induced hypertension)

- **Ethical clearance:** The Experimental study was conducted on Albino Wistarrats after approval from the Institutional Animal Ethical Committee. Experimental study was conducted in Institute of Biomedical and Industrial Research, Vidyadhar Nagar, Jaipur. We had taken IAEC approval from ethical committee of institute of biomedical and industrial research. CPCSEA Approval No: 1737/PO /RC/14/CPCSEA, IAEC No:ibir/cpcsea/iaec/2017 /7/15.

**Methods:**

- **Preparation of aqueous extract: (Test drug)**

Dried plant material was ground to coarse powder 20gm. drug was extracted with 200ml. of water using Soxhlet apparatus (Hot extraction method) till 72 hrs. At the end of extraction the extract was concentrated and solvent was totally evaporated in a petridish with the help of water bath. The thick and sticky paste thus obtained was stored in air tight container at room temperature till further use. And this extract was used as test drug.

**A. Oral Acute Toxicity Study**

Acute toxicity was done according to OECD guideline 423 ANNEX 2c

**Anti-hypertensive activity:- CdCl2Induced hypertension in albino wistar rats**

A total of 60 albino wistarrats (both sex) wereused in 4 month to 6 month age range and having weight about 70-140gm.in this study. These were divided into 5 groups of 6 rats each in both model. They were kept in separate cages after marking for 24hours prior to study. They were given food and water ad libitum.

**Marking of albino wistar rat for identification:**

The albino rat was marked with Picric acid in each group as H, B, T, HB, BT and HT where:-

- H stands for Head of albino rat
- B stands for Back of albino rat
- T stands for Tail of albino rat
- HB stands for Head back of albino rat
- BT stands for Back Tail of albino rat
- HT stands for Head tail of albino rat.

- **Test drug dose Calculated for Experimental Albino Rats –**

Test dose was derived from Acute Toxicity Study.

**Table 5: Drug used for the Treatment of induced hypertension in Animal Groups**

| Group | No. of rats | Intervention | Duration |
|---|---|---|---|
| A | 6 | 1% CMC solution | 4 week |
| B | 6 | Test drug (Aqueous Extract of whole plant of *Nardostachysjatamansi)* | 4 week |
| C | 6 | Verapamil (15 mg/kg/day, P.O.) | 4 week |

The dose for experimental study was calculated by extrapolating the human dose to animal's dose based on the body surface area ratio. Hence the suitable dose for albino rat was calculated by referring to the table of **Paget and Barne's** i.e.

✓ Hypothetical Human dose of extract of formulation = 1000mg twice daily

✓ Conversion factor (Human to Albino rat) = 0.018

✓ 18 albino wistar rats of both sexes of 3 groups (A, B and C) were administered 1mg/kg/day Intra peritoneal (0.1% $CdCl_2$ solution) $CdCl_2$ for 15 days for inducing hypertension. On day $16^{th}$ rats were weighed & their blood pressure was measured by non- invasive BP system for rodents and test sample had been administer for 30 days orally, after every week systolic blood pressure and diastolic blood pressure of each rats were measured.

Measurement using tail cuff method, rats were trained for at least one week until the BP was steadily recorded with minimal stress and restrain. Systolic and diastolic BP was measured weekly for 4 weeks by indirect non-invasive tail-cuff method.

**1.6 STATISTICAL ANALYSIS:**

The observed data had been recorded and presented in tabular format. These data had been analyzed to establish patterns of results. The data had been subjected to ANOVA test to assess the statistical significance of the findings

**Acute oral toxicity study: (As per the OECD Guidelines 423 ANNEX 2c)-**Acute Oral Toxicity (OECD GUIDELINE 423):- Observation Report of test drug

**Table 6: Observations of toxicity at dose 2000mg/kg/body wt. of test drug**

| Observation | 30min. | 4hr. | 24hr. | 48hr. | 1week | 2week |
|---|---|---|---|---|---|---|
| Skin and Fur | Normal | Normal | Normal | Normal | Normal | Normal |
| Eyes | Normal | Normal | Normal | Normal | Normal | Normal |
| Mucous Membrane | Normal | Normal | Normal | Normal | Normal | Normal |
| Salivation | Normal | Normal | Normal | Normal | Normal | Normal |
| Lethargy | Nil | Nil | Nil | Nil | Nil | Nil |
| Sleep | Normal | Normal | Normal | Normal | Normal | Normal |
| Coma | Nil | Nil | Nil | Nil | Nil | Nil |
| Convulsions | Nil | Nil | Nil | Nil | Nil | Nil |
| Tremors | Nil | Nil | Nil | Nil | Nil | Nil |
| Diarrhoea | Nil | Nil | Nil | Nil | Nil | Nil |
| Morbidity | Normal | Normal | Normal | Normal | Normal | Normal |
| Mortality | Nil | Nil | Nil | Nil | Nil | Nil |

**Hematological test:**

**Table 7: Hematological test report:**

| Sr. No. | Hematological Parameters | 300 mg/kg | 2000 mg/kg | Normal Range |
|---|---|---|---|---|
| 1. | Haemoglobin | 14.5 | 13.7 | 11.5-16.1 |
| 2. | WBC | 6.9 | 6.4 | 6.6-12.6 x $10^3$/$mm^3$ |
| 3. | RBC | 8.4 | 6.98 | 6.76-9.75 x $10^6$/ $mm^3$ |
| 4. | Neutrophils | 2.56 | 2.27 | 1.77-3.38 x$10^3$/ $mm^3$ |
| 5. | Lymphocytes | 9.1 | 8.4 | 4.78-9.12 x $10^3$/ $mm^3$ |
| 6. | Eosinophils | 0.04 | 0.04 | 0.03-0.08 x $10^3$/ $mm^3$ |
| 7. | Monocytes | 0.02 | 0.01 | 0.01-0.04 x $10^3$/ $mm^3$ |
| 8. | Basophiles | 0.0 | 0.0 | 0.00-0.03 x $10^3$/ $mm^3$ |
| 9. | Platelets | 396 | 421 | 150-460 x $10^3$/mol |

**$CdCl_2$ induce Hypertension in Albino Rats**

**Table 8: Diastolic Pressures of different groups at different time interval**

| Systolic | 1 day | 8 day | 15 day | 23 day | 30 day | 37 day |
|---|---|---|---|---|---|---|
| | Mean± SEM | Mean± SEM | Mean± SEM | Mean± SEM | Mean± SEM | Mean± SEM |
| Group A | 138±1.862 | 139.83±1.364 | 140.67±1.687 | 139.67±2.459 | 140.67±1.563 | 138.83±1.772 |
| Group B | 139.83±2.056 | 133±0.601 | 124.67±1.116 | 123.17±1.576 | 120.5±1.118 | 142±1.36 |
| Group C | 140.83±1.195 | 121.17±1.222 | 119.5±1.204 | 120.17±0.98 | 119.83±0.792 | 150.5±6.854 |

**Table 9: Systolic Pressure of different groups at different time interval**

| Diastolic | 1 day | 8 day | 15 day | 23 day | 30 day | 37 day |
|---|---|---|---|---|---|---|
| | Mean± SEM | Mean± SEM | Mean± SEM | Mean± SEM | Mean± SEM | Mean± SEM |
| Group A | 108.67±2.246 | 109±2.671 | 111.17±2.496 | 111±3.13 | 106.5±1.057 | 107.83±1.922 |
| Group B | 107.17±1.352 | 93±1.125 | 84.33±1.282 | 82±0.816 | 81±0.856 | 107.33±1.745 |
| Group C | 103.5±2.432 | 81±0.966 | 79.83±0.601 | 78.5±0.992 | 81.17±1.138 | 106±1.713 |

**Table 10: Comparison of hypertension between group A, group B & group C**

| Dunnett's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant? | Summary | Adjusted P Value |
|---|---|---|---|---|---|
| 8 day | | | | | |
| Group A vs. Group B | 6.83 | 0.2238 to 13.44 | Yes | * | 0.0404 |
| Group A vs. Group C | 18.66 | 12.05 to 25.27 | Yes | **** | 0.0001 |
| 15 day | | | | | |
| Group A vs. Group B | 16 | 9.394 to 22.61 | Yes | **** | 0.0001 |
| Group A vs. Group C | 21.17 | 14.56 to 27.78 | Yes | **** | 0.0001 |
| 23 day | | | | | |
| Group A vs. Group B | 16.5 | 9.894 to 23.11 | Yes | **** | 0.0001 |
| Group A vs. Group C | 19.5 | 12.89 to 26.11 | Yes | **** | 0.0001 |
| 30 day | | | | | |
| Group A vs. Group B | 20.17 | 13.56 to 26.78 | Yes | **** | 0.0001 |
| Group A vs. Group C | 20.84 | 14.23 to 27.45 | Yes | **** | 0.0001 |
| 37 day | | | | | |
| Group A vs. Group B | -3.17 | -9.776 to 3.436 | No | ns | 0.5801 |
| ension Group A vs. Group C | -11.67 | -18.28 to -5.064 | Yes | **** | 0.0001 |

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