Nardostachys jatamansi: cardioprotective and hypolipidemic herb
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ABSTRACT

Nardostachys jatamansi belongs to the family Valerianaceae. It is commonly used as a folk medicine for hypolipidemic activity. This made us to evaluate the hypolipidemic and cardioprotective activity in isoproterenol and triton WR 1339 administered diseased animals. Pretreatment of isoproterenol administered animals with 50 mg/kg b.wt, of Nardostachys jatamansi was found to exhibit a better antioxidant and hypolipidemic activity. Mild changes in cardiac markers reveal that the dose of Nardostachys jatamansi should be increased. In triton WR 1339 induced hyperlipidemic rats, administration of 1000 mg/kg b.wt. of extract for seven days showed the hypolipidemic activity.

Keywords: Isoproterenol, Triton WR 1339, cardiac markers, lipid profile, antioxidant

INTRODUCTION

Cardiovascular disease is the leading cause of death in both developed and developing countries (1). Myocardial infarction is the rapid development of myocardial necrosis caused by critical imbalance between the oxygen supply and the demand of the myocardium. A better understanding of the processes involved in myocardial infarction has stimulated the search for new drugs which could limit the myocardial injury. The major abnormalities noticed in myocardial infarction are lipidemia, peroxidation, and loss of plasma membrane integrity. In recent times many medicinal plants such as Picrorrhiza kurroa (2), Ocimum sanctum (3), Inula racemosa (4), Ginkgo biloba (5), Zingiber officinale (6), etc., continue to provide valuable therapeutic agents for the treatment of cardiac diseases both in modern medicine and by the traditional system throughout the world. So, this study is undertaken to evaluate the cardioprotective effect of herb Nardostachys jatamansi (NJ) Jones DC belongs to the family Valerianaceae of plant taxa. It is used as folk medicine for hypolipidemic activity, hepatoprotective and also as a diuretic and antiseptic agent. The same has been reported in Unani system of medicine. The rhizomes of Jatamansi are used as a bitter tonic stimulant and anti-spasmodic as well as to treat epilepsy, hysteria, palpitations and convulsions (7). The decoction of the root is used in mental disorders, insomnia and, disorders of the blood and the circulatory system (8). NJ exhibits anti-lipid peroxidative property in iron induced lipid peroxidation (9). Various pharmacological activities like hepatoprotective, preventing cerebral ischemia and anti-arrhythmic activity has been reported earlier (8). Phytochemical investigation has shown the presence of various sesquiterpenes such as Jatamansic acid and Jatamansone, lignans and neolignans have been reported to be present in the roots of the plant. In addition, volatile oils like jatamansic oil and other chemical substances have been isolated from various fractions of the roots and the rhizomes of the herb (10). The protective efficacy of NJ on mitochondrial respiration and lysosomal hydrolases during doxorubicin induced myocardial injury has been reported earlier (11). In this study, we have evaluated the hypolipidemic activity of NJ in Triton WR 1339 induced hyperlipidemic rats and cardioprotective effect in isoproterenol induced cardiotoxic rats.

MATERIALS AND METHODS

Collection of plant material
Roots of NJ were collected from Madurai, Tamilnadu, India. They were identified and authenticated at Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur, Tamilnadu, India and the specimen of the same is maintained. The voucher number is 0098.

Preparation of extract
The cleaned, shade dried roots of NJ was coarsely powdered by using pulverizer. 1.0 kg of coarsely powdered plant material was soaked in 5.0 liter of solvent mixture containing ethanol:water (70:30) for one week. The filtered extract was concentrated using water bath. The final traces of solvent were removed under reduced pressure and maintained at 4°C. The yield of extract was calculated as 4.57 %.

Chemicals and Reagents
Tyloxapol (Triton WR 1339) and isoproterenol hydrochloride (ISO) were purchased from Sigma chemicals, USA. All other chemicals used were of analytical grade.
**Experimental animals**

Experimental animals were maintained according to the Institutional Animal Ethical Committee (IAEC), regulated by the committee for the purpose of control and Supervision of Experiments on Animals (CPCSEA), Animal Ethical Committee No. 7/SASTRA/IAEC/RPP. Male Wistar albino rats weighing 220-230 g were purchased from Centre for Advanced Research In Indian System of Medicine (CARISM), SASTRA University, Tamil Nadu, India. The animals were housed in polypropylene cages maintained in controlled temperature and 12 hr light-dark cycle. The animals were fed with pellets and water ad libitum.

**Experimental protocols**

**Experimental protocol for cardioprotective effect in isoproterenol induced cardiotoxicity**

Four groups of animals were selected for the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal 5 % Tween 80 in saline</td>
</tr>
<tr>
<td>Group 2</td>
<td>5 % Tween 80 in saline + Isoproterenol (ISO) (85 mg/kg b.wt. s.c. in saline)</td>
</tr>
<tr>
<td>Group 3</td>
<td>50 mg/kg b.wt. of extract dissolved in 5 % Tween 80 in saline</td>
</tr>
<tr>
<td>Group 4</td>
<td>50 mg/kg b.wt. of extract dissolved in 5 % Tween 80 in saline + ISO (85 mg/kg b.wt. s.c. in saline)</td>
</tr>
</tbody>
</table>

Animals were pre-treated with extract or vehicle for 30 days. On 29th day 1 hr after the administration of extract or vehicle, ISO was administered subcutaneously. Two doses of ISO (85 mg/kg b.wt.) were administered at 24 hours interval. After administration of 2nd dose of ISO, animals were fasted for 24 hours and at the end of 24th hour (i.e. 48 hours after the administration of 1st dose of ISO) the animals were sacrificed. Blood was collected in the tubes containing EDTA for the separation of plasma and hearts were separated and washed immediately in cold saline. The weight of the hearts were noted down and then, homogenized in 0.1 M Tris HCl buffer of pH 7.4. Various biochemical parameters includes Creatine kinase (CK) (12), Lactate Dehydrogenase (LDH) (13), Aspartate transaminase (AST) (14), Lipid peroxidation (LPO) (15), Glutathione peroxidase (GPx) (16), Reduced Glutathione (GSH) (17) were analyzed. Plasma total cholesterol and High Density Lipoprotein – Cholesterol (HDL-C) concentrations were determined using enzymatic kits from Randox Laboratories Ltd., United Kingdom (18). Plasma Triglycerides (TGL) concentrations were determined by Foster (19). HDL-C was analyzed after precipitation of apo B-containing lipoproteins with dextran sulfate (20). Very Low Density Lipoprotein (VLDL) is TGL/5. Low Density Lipoprotein - Cholesterol (LDL-C) concentrations were then determined using the Friedewald equation (21).

**Experimental protocol for Hypolipidemic effect in Triton WR 1339 induced hyperlipidemia (22)**

Five groups of animals were selected for the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Normal 5 % Tween 80 in saline</td>
</tr>
<tr>
<td>Group 2</td>
<td>10 % Triton WR 1339 (400 mg/kg b.wt. i.p. in saline)</td>
</tr>
<tr>
<td>Group 3</td>
<td>Atorvastatin (10 mg/kg b.wt. p.o.) + 10 % Triton WR 1339 (400 mg/kg b.wt. i.p. in saline)</td>
</tr>
<tr>
<td>Group 4</td>
<td>500 mg/kg b.wt. of extract p.o. + 10 % Triton WR 1339 (400 mg/kg b.wt. i.p. in saline)</td>
</tr>
<tr>
<td>Group 5</td>
<td>1000 mg/kg b.wt. of extract p.o. + 10 % Triton WR 1339 (400 mg/kg b.wt. i.p. in saline)</td>
</tr>
</tbody>
</table>

Administration of the test drug should begin 4 days before the administration of Triton WR – 1339 and it should be continued up to 7 days. On 5th day Triton WR – 1339 should be injected 30 minutes after the administration of drug. The animals were fasted for 3 hours before administration of Triton WR – 1339 and the fasting was continued up to 48 hours after administration of Triton WR – 1339. 48 hours after the administration of Triton WR – 1339 blood was drawn from opthalmic plexus vein and various biochemical parameters like total cholesterol (18), LDL-C (21), VLDL (TGL/5) and TGL (19) in plasma were analysed.

**Statistical analysis**

Statistical analysis was carried out using SPSS software version 12.0. All the data are represented in Mean ± SD. Significant difference was evaluated using One Way ANOVA with Duncan Multiple Range Test (DMRT). p<0.05 was considered as significant difference.

**RESULTS**

The cardiac markers was found to be decreased significantly (p<0.05) in plasma and increased in heart homogenate of diseased rats. Treating animals with extract for 30 days was found to decrease the release of CK in to the plasma significantly (p<0.05, Table 1). Significant differences had not been observed in other parameters. The extent of TBARS generation was found to be increased in diseased rats (p<0.05, Table 2) in both plasma and heart homogenate. Likewise the antioxidants level was found to be decreased significantly in diseased conditions. Pre-treating animals with extract was found to return back all the enzyme levels to the normal level. Total cholesterol, LDL, VLDL and TGL levels were found to be increased and HDL was observed to be decreased in diseased rats. Pre-treating animals with extract was found to return the lipid profile level to a normal condition (p<0.05). In Triton WR 1339 administered diseased animals lipid profile was found to be increased significantly (p<0.05, Table 4). NJ (500 mg/kg b.wt.) was found to decrease the level of cholesterol and LDL significantly (p<0.05, Table 4). Significant differences had not been observed in the TGL and VLDL level. Whereas, pre-treating animals with 1000 mg/kg b.wt. of extract was found to decrease the level of entire lipid profile. The effect of NJ was found to be comparatively less than that of atorvastatin.

**DISCUSSION**

ISO-induced cardiac toxic serves as a standardized model to study the beneficial effects of many drugs in cardiac function. ISO, in large dose induces morphological and functional alterations in the...
Studies have shown that 50 mg/kg/b.wt, of NJ is shown to offer mild protection against myocardial injury induced by ISO in rats. Increasing the dose of extract might have been responsible for these total antioxidant activity. The increased level of antioxidants in treatment might be responsible for the decreased level of thiobarbituric acid Reactive Substances (TBARS) significantly (p<0.05, Table 2) against diseased rats. Reduced glutathione and glutathione peroxidase level of Group IV is found to be increased against Group II animals. The increased level of antioxidant activity. The increased level of antioxidants in treatment might be responsible for the decreased level of TBARS in Group IV animals. The antioxidant as well as free radical scavenging activity of the phytochemical constituents present in NJ extract might have been responsible for these total antioxidant activity (29,30). All lipid parameters are found to be increased in ISO administered rats against normal animals (p<0.05, Table 3), Similar results have been observed by Kumar (31). Extract treatment is found to be decreased significantly against diseased rats (p<0.05, Table 3). This heart leading to myocardial necrosis (23). ISO also produces excessive production of free radicals resulting from oxidative metabolism of catecholamine. Although cardiotoxicity occurs primarily via adrenoceptor activation (24), there is increasing evidence that it may also occur through oxidative mechanisms (25). Administration of ISO causes an increased elevation of all cardiac markers like CK, LDH and AST in diseased rats against normal rats. This might be due to the membrane damage (26). In the present study, NJ treatment is found to decrease the level of CK in plasma. But other cardiac markers are not found to be decreased significantly in both plasma and heart homogenate. Slight changes in values reveal that they are trying to exhibit their activity. Increasing the dose of extract may exhibit better activity. NJ’s mild cardio-protection action on the cell membranes has been furnished through significant reduction in the decrement of CK release. CK is considered as a better cardiac marker when compared to other markers like LDH, AST (27). It could be inferred from the observations that 50 mg/kg/b.wt, of NJ is shown to offer mild protection against myocardial injury induced by ISO in rats. Increasing the dose of extract may protect the heart from drastic cardio-toxicity developed by ISO. Oxidative stress resulting from increased production of free radicals associated with decreased levels of antioxidants in the myocardium, plays a major role in diseases such as ischemic heart disease, atherosclerosis, congestive heart failure, cardiomyopathy and arrhythmia (28). NJ treatment is found to decrease the elevated Thiobarbituric acid Reactive Substances (TBARS) significantly (p<0.05, Table 2) against diseased rats. Reduced glutathione and glutathione peroxidase level of Group IV is found to be increased against Group II animals might be due to the antioxidant activity. The increased level of antioxidants in treatment might be responsible for the decreased level of TBARS in Group IV animals. The antioxidant as well as free radical scavenging activity of the phytochemical constituents present in NJ extract might have been responsible for these total antioxidant activity (29,30). All lipid parameters are found to be increased in ISO administered rats against normal animals (p<0.05, Table 3). Similar results have been observed by Kumar (31). Extract treatment is found to be decreased significantly against diseased rats (p<0.05, Table 3). This

### Table 1 Effect of Nardostachys jatamansi on cardiac markers in isoproterenol induced oxidative stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH (heart)</th>
<th>CK (heart)</th>
<th>GOT (heart)</th>
<th>LDH (plasma)</th>
<th>CK (plasma)</th>
<th>GOT (plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.7 ± 0.04 a</td>
<td>2369.4 ± 4.4 b</td>
<td>2.3 ± 0.03 b</td>
<td>0.2 ± 0.004 a</td>
<td>4.5 ± 0.03 a</td>
<td>0.05 ± 0.0003 a</td>
</tr>
<tr>
<td>2</td>
<td>1.4 ± 0.06 a</td>
<td>1745.3 ± 3.3 a</td>
<td>1.4 ± 0.017 a</td>
<td>0.44 ± 0.003 b</td>
<td>8.3 ± 0.03 c</td>
<td>0.08 ± 0.0009 b</td>
</tr>
<tr>
<td>3</td>
<td>2.5 ± 0.04 a</td>
<td>2217.1 ± 1.4 b</td>
<td>2.2 ± 0.042 b</td>
<td>0.21 ± 0.009 a</td>
<td>4.5 ± 0.04 a</td>
<td>0.05 ± 0.0001 a</td>
</tr>
<tr>
<td>4</td>
<td>1.8 ± 0.7 b</td>
<td>178.9 ± 8.4 a</td>
<td>1.4 ± 0.031 a</td>
<td>0.46 ± 0.007 b</td>
<td>7.3 ± 0.06 b</td>
<td>0.073 ± 0.0001 b</td>
</tr>
</tbody>
</table>

*Values are Mean ± SD. Values not sharing common alphabets are different significantly at p<0.05. Unit - LDH–µg of pyruvate liberated/min/mg of protein; CK–µg of phosphorous liberated/min/mg of protein.

### Table 2 Effect of Nardostachys jatamansi on antioxidants in isoproterenol induced oxidative stress

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO (plasma)</th>
<th>LPO (heart)</th>
<th>GSH (plasma)</th>
<th>GSH (heart)</th>
<th>GPX (plasma)</th>
<th>GPX (heart)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.08 ± 0.0005 a</td>
<td>0.62 ± 0.0007 a</td>
<td>4.6 ± 0.019 b</td>
<td>124.6 ± 6.0 b</td>
<td>3.6 ± 0.06 b</td>
<td>3.4 ± 0.08 b</td>
</tr>
<tr>
<td>2</td>
<td>0.17 ± 0.0002 b</td>
<td>0.92 ± 0.0011 b</td>
<td>2.6 ± 0.08a</td>
<td>83.6 ± 2.5 a</td>
<td>2.7 ± 0.1a</td>
<td>2.2 ± 0.04 a</td>
</tr>
<tr>
<td>3</td>
<td>0.08 ± 0.0001 a</td>
<td>0.64 ± 0.0005 a</td>
<td>4.6 ± 0.018 b</td>
<td>121.7 ± 9.3 b</td>
<td>3.9 ± 0.3 b</td>
<td>3.5 ± 0.05 b</td>
</tr>
<tr>
<td>4</td>
<td>0.09 ± 0.0002 a</td>
<td>0.79 ± 0.0002 ab</td>
<td>4.2 ± 0.06 b</td>
<td>126.6 ± 4.3 b</td>
<td>3.6 ± 0.66 b</td>
<td>4.0 ± 0.07 b</td>
</tr>
</tbody>
</table>

*Values are Mean ± SD. Values not sharing common alphabets are different significantly at p<0.05. Unit - LPO-nM of MDA/mg of protein; GSH-µg of GSH/mg of protein; GPX-µg of GPX/min/mg of protein.

### Table 3 Effect of Nardostachys jatamansi on plasma lipid profile in isoproterenol induced oxidative stress

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>TGL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74.1 ± 4.1 a</td>
<td>20.3 ± 0.7 b</td>
<td>40.4 ± 1.2 a</td>
<td>13.4 ± 0.2 a</td>
<td>66.8 ± 4.6 a</td>
</tr>
<tr>
<td>2</td>
<td>95.2 ± 2.7 b</td>
<td>14.8 ± 0.4 a</td>
<td>62.8 ± 1.3 c</td>
<td>17.6 ± 0.3 b</td>
<td>87.8 ± 2.3 b</td>
</tr>
<tr>
<td>3</td>
<td>71.6 ± 5.0 a</td>
<td>20.5 ± 2.4 b</td>
<td>37.28 ± 2.6 a</td>
<td>13.8 ± 0.7 a</td>
<td>69.1 ± 4.4 a</td>
</tr>
<tr>
<td>4</td>
<td>86.8 ± 3.6 ab</td>
<td>16.5 ± 1.4 ab</td>
<td>50.04 ± 3.2 b</td>
<td>15.3 ± 0.4 ab</td>
<td>76.3 ± 4.6 ab</td>
</tr>
</tbody>
</table>

*Values are Mean ± SD. Values not sharing common alphabets are different significantly at p<0.05.

### Table 4 Effect of Nardostachys jatamansi on serum lipid profile in Triton WR 1339 induced hyperlipidemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
<th>TGL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61.25 ± 2.51 a</td>
<td>40.8 ± 5.2 a</td>
<td>16.2 ± 2.3 a</td>
<td>50.21 ± 3.36 a</td>
</tr>
<tr>
<td>2</td>
<td>850.2 ± 8.3 d</td>
<td>699.84 ± 10.3 d</td>
<td>120.06 ± 8.3 d</td>
<td>600.3 ± 10.2 d</td>
</tr>
<tr>
<td>3</td>
<td>473.5 ± 7.5 b</td>
<td>312.3 ± 5.6 b</td>
<td>105.02 ± 5.6 b</td>
<td>525.1 ± 12.4 b</td>
</tr>
<tr>
<td>4</td>
<td>670.3 ± 73.3 c</td>
<td>473.7 ± 7.5 c</td>
<td>120.5 ± 2.5 d</td>
<td>607.5 ± 12.5 d</td>
</tr>
<tr>
<td>5</td>
<td>650.6 ± 47.8 c</td>
<td>449.8 ± 47.7 c</td>
<td>115.0 ± 2.8 c</td>
<td>575.0 ± 14.4 c</td>
</tr>
</tbody>
</table>

*Values are Mean ± SD. Values not sharing common alphabets are different significantly at p<0.05.
might be due to the hypolipidemic effect of NJ.

NJ is reported to consist of oil. (10) Sudhahar (32) has explained that the triterpenes can markedly decrease the myocardial levels of Total cholesterol and TGL. Incorporation of sterols through NJ may also lessen the incorporation of dietary and biliary cholesterol into micelles, thereby, lowering cholesterol absorption in the intestines and decreased the plasma lipid profile. LPO is an important mechanism of modifying LDL. (33). The data from our study clearly indicate that the ISO administered rats have both the hyperlipidemic condition and an increase in the levels of lipid peroxides which pays the better way for the formation of ox-LDL. Heinecke (34) has explained that the action of antioxidants is commonly linked to the inhibition of lipoprotein oxidation. The underlying assumption is that the antioxidant activity of NJ may protect the heart from oxidative stress and exhibits hypolipidemic activity. In conclusion, to increase the efficacy of NJ in ISO induced cardio-toxicity the dose of the extract should be increased.

To reconfirm the hypolipidemic effect, efficacy of the extract with higher dose and shorter duration, the NJ extract is evaluated in Triton WR 1339 induced hyperlipidemic rats. Triton WR 1339, a surface active agent, widely used for a number of different aims, in particular, in rats it has been used for screening natural or chemical hypolipidemic drugs (35,36). In the Triton WR 1339 induced hyperlipidemic rats, the extract dose is increased 10 times than that of ISO induced cardio-toxic study and the duration of study is fixed as 7 days. Schurr (37) and Hicham (38) have demonstrated that parenteral administration of a dose of Triton WR 1339 to adult rats induced hyperlipidaemia. In our present study, intra peritoneal injection of Triton WR 1339 causes increased level of lipid profile in plasma. Treatment with NJ is found to decrease the level of lipid profile like cholesterol, TGL, LDL and VLDL significantly (p<0.05, Table 4). Though significant difference has not been observed in cholesterol and LDL level by increasing the dose of NJ, significant difference had been observed in TGL level. These results reveal that plateau region had been observed at 500 mg/kg b.wt. of extract treatment in decreasing cholesterol and LDL level. The decrement of cholesterol and LDL by NJ is found to be lesser than that of standard drug treatment. But as reported in the ISO induced cardiotoxic study NJ extract is antioxidant in nature and hypolipidemic with lower dose of extract in sub-chronic study. The efficacy of NJ should be compared with that of fibronectin or other modern drugs with potent hypo-triglycerideemic effect.

CONCLUSION

70% hydroalcoholic extract of NJ (50 mg/kg b.wt.) protect heart from ISO induced cardiotoxicity. The cardio-protective activity of NJ is due to the antioxidant and hypolipidemic activity. To observe a better cardio-protection in ISO administered animals, higher dose of extract is required. NJ at 500 mg/kg b.wt. is also found to decrease the lipid profile significantly within seven days in Triton WR 1339 administrated hyperlipidemic rats. Thus, from the present study it has been concluded that NJ is hypolipidemic as well as cardio-protective.

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REFERENCES


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