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# Biochemical study on the protective potential of *Nardostachys jatamansi* extract on lipid profile and lipid metabolizing enzymes in doxorubicin intoxicated rats

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*Received August 18, 2006, accepted September 29, 2006*

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*Pharmazie 62: 382–387 (2007)* *doi: 10.1691/ph.2007.5.6678*

*Nardostachys jatamansi* is a medicinally important herb of Indian origin used for centuries in Ayurvedic and Unani systems of medicine for the treatment of various ailments. The aim of the present work is to evaluate the effect of ethanolic extract of *Nardostachys jatamansi* rhizomes on doxorubicin induced myocardial injury with respect to lipid metabolism in serum and heart of Wistar albino rats. Altered lipid metabolism alters the cardiac function which is mainly due to changes in the property of the cardiac cell membrane. Doxorubicin exhibits cardiotoxicity by inhibition of fatty acid oxidation in the heart. The rats treated with a single dose of doxorubicin (15 mg/kg) intraperitoneally showed an increase in serum and cardiac lipids (cholesterol, triglycerides, free fatty acids and phospholipids), along with a significant rise in serum low density lipoproteins (LDL), very low density lipoproteins (VLDL) and drop in high density lipoproteins (HDL) levels, resulting in alteration of serum and cardiac lipid metabolizing enzymes. Pretreatment with a extract of *Nardostachys jatamansi* (500 mg/kg) orally for seven days to doxorubicin induced rats showed a significant prevention in the lipid status with the activities of the lipid metabolizing enzymes. Histopathological observations were also in correlation with the biochemical parameters. These findings suggest that the protective and hypolipidemic effect of *Nardostachys jatamansi* against doxorubicin induced myocardial injury in rats could possibly be mediated through its anti lipid peroxidative properties.

## 1. Introduction

*Nardostachys jatamansi* commonly known as Jatamansi, Indian Nard, Balchar or Spikenard, is a small herbaceous species belonging to the Valerianaceae family (Chauhan and Nautiyal 2005). In Ayurveda, rhizomes of *N. jatamansi* are used as a bitter tonic, stimulant and antispasmodic, and to treat epilepsy, hysteria and convulsions (Bagchi et al. 1991). The decoction of the root is used in mental disorders, insomnia and disorders of blood and circulatory system (Uniyal and Issar 1969). In the Unani system of medicine this plant has been used as a hepatotonic, cardiotonic, diuretic and analgesic (Ali et al. 2000). Recent reports have shown this plant to be a potent antioxidant (Tripathi et al. 1996), neuroprotective (Salim et al. 2003), and could be used in the treatment of ischemic stroke (Green et al. 2000).

The phytochemical analysis of the plant showed the presence of alkaloids, coumarins, sesquiterpenes, lignans, neolignans and terpenoids (Chatterjee et al. 2000) which are compounds that possess hypolipidemic activity (Kirmukog et al. 1991; Kuroda et al. 1997; Silva et al. 2005). We previously reported the effect of an ethanolic extract of *N. jatamansi* on the antioxidant defense system during doxorubicin (DOX) induced cardiotoxicity (Subashini et al. 2006). Hence our present study is aimed to study the efficacy of a 95% ethanolic extract of *N. jatamansi* on lipid metabolic changes in heart due to DOX administration in rats.

A number of different hypotheses have been proposed to account for the cardiotoxic effect of DOX. These include the production of free radical species (Doroshow 1983), leading to lipid peroxidation of cardiac microsomal membranes (Limbaugh et al. 1983), the differential accumulation and retention of positively charged DOX as a result of highly negative membrane potential (Lampidis et al. 1981), an interaction with nucleic acid or nuclear components (Torti et al. 1998), and disruption of a cardiac specific program of gene expression (Arai et al. 1998).

Increased myocardial lipid accumulation and plasma lipid levels are usually associated with DOX-induced cardiomyopathy (Iliskovic and Singal 1997). It has been suggested that DOX may exert at least part of its cardiotoxicity by inhibition of fatty acid oxidation in the heart (Abdel-aleem et al. 1997), because long-chain fatty acids are the major substrates for energy production in the aerobic adult myocardium (Neely and Morgan 1974). Impaired cardiac fatty acid oxidation is usually associated with dia-

stolic dysfunction (Bordoni et al. 1999), cardiomyopathy, and congestive heart failure as a result of a deficiency in energy supply and possible accumulation of toxic intermediates of fatty acid oxidation in cardiac tissues (Sayed-Ahmed 1999). However, there have been no studies showing the effect of *N. jatamansi* extract on lipid and lipoprotein status in DOX induced myocardial injury in rats. Therefore, the present study was devised to determine the beneficial effect of *N. jatamansi* extract against DOX induced cardiomyopathic rats.

## 2. Investigations and results

### *2.1. Effect of N. jatamansi on lipoprotein fractions*

Fig. 1 shows the levels of LDL, HDL and VLDL cholesterol in the serum of control and experimental rats. DOX induction showed a significant increase in the levels of LDL and VLDL along with significant decrease in HDL cholesterol levels. Pretreatment with *N. jatamansi* in group IV rats significantly lowered LDL, VLDL and increased HDL concentrations in the serum. Oral administration of *N. jatamansi* extract alone in group III rats showed a significant increase in HDL levels when compared with group I rats.

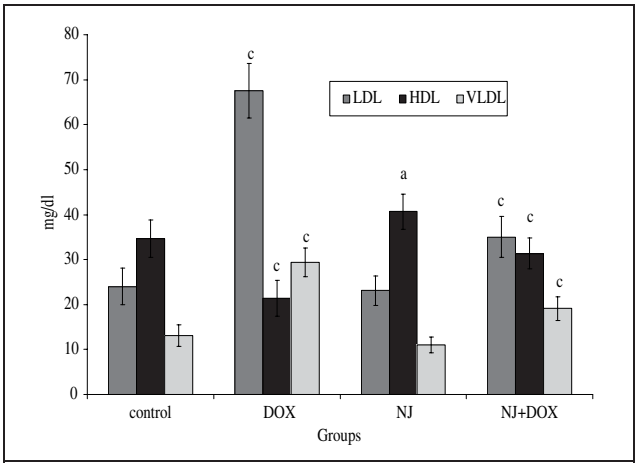


Fig. 1.: Effect of *Nardostachys jatamansi* in serum lipoprotein levels in control and experimental animals. Values are expressed as mean ± S.D for six animals in each group. The symbol represents the statistical significance. [a] $P < 0.05$, [c] $P < 0.001$. Comparisons are made between DOX and control group; DOX and *N. jatamansi* + DOX group. Units: mg/dl

### *2.2. Effect of N. jatamansi on lipid profile in serum and heart*

Tables 1 and 2 present the levels of cholesterol, triglycerides, phospholipids and free fatty acids in the serum and heart of control and experimental rats. DOX induction in group II rats showed a significant increase in the levels of cholesterol, triglycerides, phospholipids and free fatty acids when compared to control rats. *N. jatamansi* pretreated group IV rats significantly ameliorated the observed abnormalities and restored the lipid levels to near normalcy when compared to group II rats. Group III rats (*N. jatamansi* alone) did not show any significant changes when compared with group I rats.

### *2.3. Effect of N. jatamansi in serum lipid metabolizing enzymes*

Table 3 shows the activities of lipid metabolizing enzymes in the serum of the control and the experimental group. The activities of TL and CES in DOX induced group II rats were significantly increased and the activities of LPL, LCAT, and CEH were significantly decreased. *N. jatamansi* pretreatment (group IV) significantly prevented these alterations and restored the levels to near normalcy. Group III rats did not show any significant changes compared to group I rats.

**Table 3: Effect of *N. jatamansi* on serum lipid metabolizing enzymes in control and experimental group of rats**

| Parameters | Control | DOX | *N. jatamansi* | *N. jatamansi* + DOX |
|---|---|---|---|---|
| TL | $2.75 \pm 1.32$ | $4.63 \pm 1.5^{a}$ | $2.72 \pm 1.43$ | $2.69 \pm 1.44^{a}$ |
| LPL | $5.54 \pm 1.63$ | $2.66 \pm 1.35^{b}$ | $5.79 \pm 1.90$ | $5.36 \pm 1.68^{a}$ |
| LCAT | $7.64 \pm 1.67$ | $3.97 \pm 1.54^{b}$ | $8.33 \pm 2.31$ | $5.59 \pm 1.44^{b}$ |
| CES | $4.48 \pm 1.52$ | $7.43 \pm 1.38^{b}$ | $4.03 \pm 1.62$ | $5.13 \pm 1.50^{a}$ |
| CEH | $6.47 \pm 1.40$ | $3.58 \pm 1.33^{b}$ | $6.46 \pm 1.50$ | $5.85 \pm 1.53^{a}$ |

Values are expressed as mean ± S.D for six animals in each group. [a] $P < 0.05$, [b] $P < 0.01$. Comparisons are made between DOX and control group; DOX and *N. jatamansi* + DOX group. Units: mg/dl

**Table 1: Effect of *N. jatamansi* on serum lipid profile in control and experimental rats**

| Parameters | Control | DOX | *N. jatamansi* | *N. jatamansi* + DOX |
|---|---|---|---|---|
| Cholesterol | $65.42 \pm 5.50$ | $139.40 \pm 12.83^{c}$ | $62.81 \pm 5.96$ | $89.56 \pm 7.2^{c}$ |
| Triglycerides | $61.20 \pm 6.85$ | $116.57 \pm 10.70^{c}$ | $59.53 \pm 5.07$ | $73.52 \pm 6.77^{c}$ |
| Phospholipids | $57.28 \pm 5.35$ | $95.28 \pm 9.25^{c}$ | $56.41 \pm 4.74$ | $70.50 \pm 5.9^{c}$ |
| Free fatty acids | $24.16 \pm 3.64$ | $39.62 \pm 3.00^{c}$ | $23.35 \pm 3.28$ | $29.80 \pm 5.25^{c}$ |

Values are expressed as mean ± S.D for six animals in each group. [c] $P < 0.001$
Comparisons are made between DOX and control group; DOX and *N. jatamansi* + DOX group. Units: mg/dl

**Table 2: Effect of *N. jatamansi* on cardiac lipid profile in control and experimental rats**

| Parameters | Control | DOX | *N. jatamansi* | *N. jatamansi* + DOX |
|---|---|---|---|---|
| Cholesterol | $4.2 \pm 0.69$ | $6.39 \pm 1.10^{b}$ | $3.48 \pm 1.66$ | $4.2 \pm 0.69^{a}$ |
| Triglycerides | $4.58 \pm 1.14$ | $6.36 \pm 1.31^{a}$ | $4.80 \pm 0.90$ | $6.10 \pm 1.55^{a}$ |
| Phospholipids | $6.38 \pm 1.39$ | $14.59 \pm 2.17^{c}$ | $6.62 \pm 2.11$ | $9.66 \pm 1.70^{c}$ |
| Free fatty acids | $0.63 \pm 0.14$ | $1.21 \pm 0.63^{b}$ | $0.58 \pm 0.08$ | $0.74 \pm 0.12^{a}$ |

Values are expressed as mean ± S.D for six animals in each group. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.
Comparisons are made between DOX and control group ; DOX and *N. jatamansi* + DOX group. Units: mg/dl

**Table 4: Effect of *N. jatamansi* on cardiac lipid metabolizing enzymes in control and experimental group of rats**

| Parameters | Control | DOX | *N. jatamansi* | *N. jatamansi* + DOX |
|---|---|---|---|---|
| TL | 1.97 ± 0.62 | 3.46 ± 1.34[a] | 2.08 ± 1.10 | 2.10 ± 1.22 [a] |
| LPL | 14.36 ± 1.58 | 9.28 ± 1.5[c] | 13.50 ± 1.49 | 12.59 ± 1.58[c] |
| LCAT | 40.46 ± 4.72 | 26.48 ± 3.14[c] | 42.42 ± 4.84 | 35.88 ± 2.40[c] |
| CES | 8.46 ± 1.43 | 13.46 ± 2.29[c] | 8.65 ± 1.29 | 9.56 ± 1.16[c] |
| CEH | 3.11 ± 1.39 | 5.21 ± 1.40[b] | 2.87 ± 1.06 | 3.64 ± 1.10[a] |

Values are expressed as mean ± S.D for six animals in each group. [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$.
Comparisons are made between DOX and control group; DOX and *N. jatamansi* + DOX group. Units: mg/dl

### *2.4. Effect of N. jatamansi in heart lipid metabolizing enzymes*

Table 4 shows the activities of lipid metabolizing enzymes in the hearts of the control and the experimental groups. The activities of TL, CES and CEH in the DOX induced group significantly increased and that of LPL and LCAT significantly decreased in the DOX intoxicated group. *N. jatamansi* pretreated rats (group IV) significantly restored the abnormalities of these lipid metabolizing enzymes. Treatment with *N. jatamansi* alone (group III) did not result in any significant changes compared to group I rats.

### *2.5. Histopathological changes*

The following histopathological observations were made in the heart of control and experimental groups of rats. Fig. 2 (A) shows the architecture of the normal cardiac tissue; (B) & (C) the rats given DOX elicited severe cardiac damage by pathological changes in the architecture of the heart, viz. muscle fibre damage, inflammation (D): the rats pretreated with *N. jatamansi* and DOX showed better cardioprotection as observed by the absence of adverse pathological changes.

## 3. Discussion

The anthracycline glycoside antibiotic DOX is of major importance in cancer chemotherapy. Experimental studies using DOX-induced animal's aid in the understanding of the drug's unfavorable cardiac and hepatic toxicities, and several therapeutic strategies have been evaluated to counter the adverse effects. The increase in the levels of serum and cardiac lipids due to DOX intoxication is an evidence

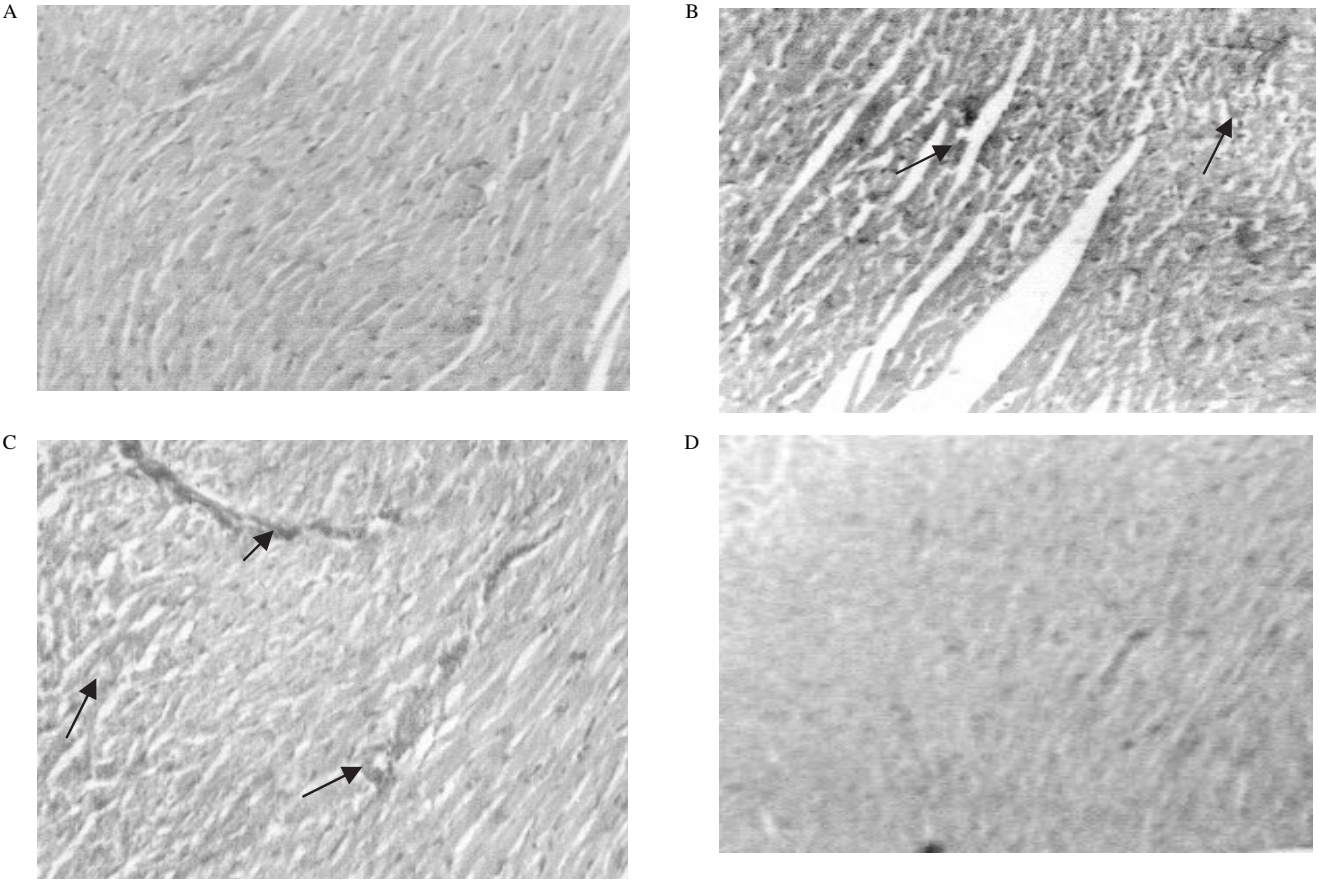


Fig. 2.: Fig. 2. (A–D). Histopathological studies of the heart tissue of control and experimental group of rats
A: Section of heart tissue from control rat showing normal architecture
B&C: The rats given DOX showed damage of muscle fibre and intense infiltration of leucocytes
D: *N. jatamansi* + DOX treated rat showing minimal changes with normal architecture of heart tissue

for its known hyperlipidemic effect (Deepa and Varalakshmi 2005). Recently, there is a global awareness for traditional systems of medicine which are studied by modern scientific methods. Medicinal plants have long been recognized as sources of new compounds with hypolipidemic activity (Muthu et al. 2005).

Lipids consist of cholesterol (HDL & LDL cholesterol), triglycerides, phospholipids and free fatty acids. Observations of significant elevations of serum cholesterol & LDL cholesterol in our studies showed that DOX reduced the rate of lipolysis, whereas HDL cholesterol was not significantly elevated. Thus, our results show that adverse effects caused by DOX such as hyperlipidemia, which is deleterious for heart function, appear to contribute to DOX-induced heart failure (Iliskovic and Singal 1997). Koutinos et al. (2002) demonstrated that the myocardial toxicity of DOX is paralleled by an increase in serum lipids especially cholesterol and triglycerides. High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular damage (Slater and White 1996). An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the decreased myocardial contractibility, arrhythmias and cell death that follow coronary artery occlution (Katz and Messinco 1981).

Lipid peroxides also play a pivotal role in myocardial damage during DOX induction (Horenstein et al. 2000). Lipid peroxidation usually begins with the removal of an hydrogen atom from an unsaturated fatty acid resulting in lipid radicals which ultimately lead to the accumulation of lipids in cardiac tissue. The cardiac muscle generally utilizes fatty acid as the major source of energy of the total oxygen consumption; 60–90% is utilized to oxidize fatty acid under aerobic condition. Under anoxic conditions, the cardiac muscle is not in a position to oxidize the available fatty acids, as a result of which there is an increase in the levels of these acids and long chain acyl CoA derivatives (Whitmer et al. 1978).

The levels of free fatty acids increase upon DOX administration (Hong et al. 2002). The liberated free fatty acids are taken up by the myocardium for its energy requirement and the excess is used for the synthesis of glycerides (Heindel et al. 1975). DOX as outlined previously increases peroxidation of the membrane phospholipids. This will lead to the release of free fatty acids mainly due to the action of phospholipase$A_2$ (Chein et al. 1980). There may be a self perpetuating cycle whereby part of the cell membrane is disrupted by the degrading phospholipids due to the action of phospholipases. There is increased peroxidation of polyunsaturated fatty acids, which is recognized as one of the possible mechanism for the genesis of membrane injury in the myocardium. The extract of *N. jatamansi* has been shown to be effective in free radical-induced lipid peroxidation by lead and to have protective activity in the major organs including the heart (Subashini et al. 2000).

The present study showed a decrease in the activity of LCAT in serum and LPL in the heart which might be due to the heart lipid peroxide levels in DOX induced rats that results in the inactivation of these enzymes (Geetha et al. 1990). The reduced activity of LPL in heart might be due to the low degradation of lipoprotein (Lespine et al. 1997). It has been suggested that a defective secretion of LPL contributes to the reduced lipolytic activity, thus resulting in increased triglycerides and phospholipids. The decreased LPL activities in plasma and tissues are in concert with the increased triglycerides in the DOX group observed herein. Increased levels of TG were observed in doxorubicin treated rats and such a state is also reported in association with cardiovascular disturbances (Freedman et al. 1988). Pretreatment of rats with *N. jatamansi* extract showed improved LPL activity, thereby restoring near normal TG levels.

Alteration in lipid metabolism directly reflects the composition of lipoproteins. The present study showed an increase in LDL and VLDL fraction and a decrease in HDL upon DOX induction. LDL is the major cholesterol carrier in the blood stream. It is well established that the cholesterol deposits in the arteries stem primarily from plasma LDL and that increased levels of plasma LDL correlate with an increased risk for atherosclerosis. Various lines of research provide strong evidence that LDL becomes oxidized *in vivo* and that oxidized LDL is involved in the formation of atherosclerotic lesions (Steinberg et al. 1989). Elevated triglycerides are often seen with lower HDL levels and this has been associated with an increased cardiovascular risk. Low HDL is an independent risk factor and is inversely related to cardiovascular disease incidence, consistent with its putative role in cholesterol removal. There is strong evidence from several studies that extent of reduction in the incidence of coronary heart diseases is related to the magnitude of reduction in LDL and VLDL cholesterol levels. The present study shows that administration of *N. jatamansi* reduces the levels of LDL and VLDL and also elevates the cardioprotective HDL cholesterol by its inhibitory effect on lipid peroxidation chain reaction (Tripathi et al. 1996) thereby reducing triglyceride levels. A previous study reported that ethanolic extract of *N. jatamansi* (whole plant) feeding has been found to increase HDL-cholesterol/cholesterol ratio (Rao et al. 2005) which well concurs with our present study. HDL is the main substrate for LCAT that results in cholesterol esterification and incorporation (Deepa and Varalakshmi 2005). The diminished levels of cardiac LCAT in DOX induced rats may be due to the low levels of HDL cholesterol. By the increase in HDL levels in *N. jatamansi* pretreated DOX induced group support the increased cardiac LCAT activity.

The elevated total cholesterol concentrations in DOX induced group might be due to the increased activity of CES and CEH (Roh et al. 1993) which may be related to cholesterol accumulation in cardiac tissues. The increase in cholesterol increases the membrane fluidity, regulates membrane permeability, and alters internal viscosity and also the internal chemical composition (Grundy 1986). *N. jatamansi* pretreatment showed a decrease in these enzymes in the cardiac tissue, thus preventing the accumulation of lipids by its antioxidant effects (Ali et al. 2000). As seen in the present study, DOX treatment caused significant histological changes including marked myofibril loss and inflammation. These pathological changes correlated well with the altered enzyme activities. While *N. jatamansi* extract pretreated rats, significantly ameliorated the observed abnormalities of histopathological changes to near normal morphology.

In conclusion, the antioxidant activity of *N. jatamansi* plays a possible role in preventing lipid peroxidation (Tripathi et al. 1996) thereby inhibiting lipolysis. It can be concluded from the above findings that *N. jatamansi* is a potent antioxidant and plays a significant role in stabilizing lipid metabolizing enzymes by its hypolipidemic effect thereby proving its potential to act as a cardioprotective drug.

## 4. Experimental

### 4.1. Plant materials and chemicals

Roots of *Nardostachys jatamansi* De Jones (Valerianaceae) were purchased from an recognized and licensed ayurvedic shop in Chennai (India) and were identified and authenticated by Dr. Sasikala Ethirajulu (Research Officer, Botany) in the Central Institute for Siddha (CRIS), Arumbakkam, Chennai-600 101. Doxorubicin was procured from Dabur pharmaceuticals (Doxorubicin hydrochloride-Adrim), New Delhi, India. All other chemicals used were of analytical grade.

### 4.2. Preparation of the extract

Clean roots were air dried and powdered to prepare the alcoholic extract as earlier described by Prabhu et al. (1994). One kilogram of moderately powdered roots of Jatamansi was extracted by refluxing with 95% ethyl alcohol in a Soxhlet extractor for 6–8 h. The extract was evaporated to dryness under reduced pressure and temperature using rotatory vacuum evaporator, and dried residue was stored at 4 °C. The yield of dry extract from crude powder of Jatamansi was 10%. The dried ethanolic extract was suspended in distilled water which was then administered to rats orally at an optimum dosage of 500 mg/kg body wt. This particular dosage was fixed after trying different doses for different days in the same set of rats (Subashini et al. 2006).

### 4.3. Animals

Adult male albino rats of Wistar strain, weighing approximately 120–130 g, were obtained from Tamilnadu Veterinary and Animal Science University, Chennai, India. They were acclimatized to animal house conditions, fed commercial pellet rat chow (Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. Experimental animals were handled according to the University and institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justices and Empowerment, Government of India (IAEC No.01/007/06).

### 4.4. Experimental protocols

The following groups of animals were used. The rats were divided into four groups ($n = 6$ in each group): Group I served as control; Group II and IV comprised of rats that received a single dose of doxorubicin (15 mg/kg, i.p). While group II was left untreated, group IV was subjected to *N. jatamansi* treatment (500 mg/kg) for seven days orally prior to the administration of doxorubicin; Group 3 rats received only *N. jatamansi* extract (500 mg/kg) orally for seven days, serving as drug control. As ethanolic extract of *N. jatamansi* was administered orally for seven days at the dose of 500 mg/kg prior to the induction of DOX showed maximum cardioprotective efficacy, this particular dosage was used as the optimal dosage for the study. Doxorubicin was given at the dose of 15 mg/kg i.p. as described by previous study (Nagi and Mansour 2000) on day 7 and the animals were sacrificed after 48 h of experimental period.

### 4.5. Extraction of cardiac lipids and measurement of lipids and lipid metabolizing enzymes

At the end of the experimental period, the rats were sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifugation at 3000 rpm for 10 min and analyzed for various biochemical parameters. The heart was excised immediately, rinsed in ice-cold saline, dried, weighed and the cardiac lipids were extracted with a chloroform : methanol mixture (2 : 1, v/v) according to Folch el al. (1957). Estimation of cholesterol was carried out using ferric chloride-uranyl acetate reagent (Parakh and Jung 1970), Phospholipids were estimated by digestion with perchloric acid (Fiske and Subbarow 1925), Free fatty acid was estimated using copper nitrate-triethanolamine reagent (Hron and Menahan 1981) and triglycerides (Foster and Dunn 1973). HDL, LDL and VLDL lipoproteins were fractionated by a dual precipitation technique (Burstein and Scholnick 1972). Lipid metabolizing enzymes such as total lipases (TL) (Bier 1955), lipoprotein lipase (LPL) (Schmidt 1974), lecithin cholesterol acyl transferase (LCAT) (Legraud and Guillansseav 1979), cholesterol ester synthetase (CES) (Kothari et al. 1973), and cholesterol ester hydrolase (CEH) (Kothari et al. 1970) were assayed.

### 4.6. Histopathology

Histopathological evaluation was performed on lower portion of the heart specimen fixed in 10% formalin and embedded in paraffin wax. Sections were cut at 4 μm in thickness, stained with haematoxylin and eosin and viewed under light microscopy for histological changes.

### 4.7. Statistical analysis

All the grouped data were statistically evaluated with SPSS/10 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. All these results were expressed as mean ± S.D for six animals in each group.

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