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The role of *Nardostachys jatamansi* against doxorubicin-induced toxicity in rats

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This investigation elucidated the role of free radicals in doxorubicin-induced toxicity and protection by *Nardostachys jatamansi (NJ)*. Adult male albino wistar rats were administered with doxorubicin (15 mg/kg; i.p.) and *NJ* (500 mg/kg, orally) for seven days. At the end of the experiment, following decapitation, heart and liver tissue samples were taken for histological examination, determination of malondialdehyde (MDA), glutathione (GSH) and myeloperoxidase (MPO) activity. In addition, proinflammatory cytokine (TNF-α) was assayed in plasma samples. The results reveal that doxorubicin caused a significant decrease in GSH level, significant increases in MDA level and MPO activity. Similarly, plasma cytokine level was elevated in doxorubicin group compared with the control group. On the other hand *NJ* pretreatment reversed all these biochemical indices. The results demonstrate that *NJ* extract, by balancing the oxidant-antioxidant status and inhibiting the generation of proinflammatory cytokine, protects against doxorubicin-induced oxidative organ injury.

Key words: *Nardostachys jatamansi*, doxorubicin, cytokine, glutathione, malondialdehyde, myeloperoxidase.

INTRODUCTION

Doxorubicin (DOX) is a quinine-containing anticancer antibiotic and widely used to treat different types of human neoplastic disease such as hematopoietic, lymphoblastic and solid tumors (Hassanpour et al., 2010). However, its clinical use is limited on account of its toxicity. DOX cytotoxic effects have been associated with reactive oxygen species (ROS) generated during drug metabolism. Several *in vivo* and *in vitro* studies have demonstrated that reactive oxygen metabolites including free radical species, superoxide anion (O_2−•), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) are important mediators of tissue injury (Mohamad et al., 2009). The cellular and biochemical changes involved in this process have been demonstrated. One-electron reduction of DOX leads to formation of the corresponding semiquinone free radical. In the presence of oxygen, this free radical rapidly donates its electron to oxygen to generate superoxide anion (O_2 •). The dismutation of superoxide yields hydrogen peroxide (H_2O_2). Under biological conditions, the anthracycline semiquinone or reduced metal ions such as iron reductively cleaves hydrogen peroxide to produce the hydroxyl radical which is the most reactive and destructive chemical species ever known. This ultimately leads to lipid peroxidation, causing irreversible damage of membrane structure and function (Giri et al., 2004).

An extract of the rhizomes of *Nardostachys jatamansi* mainly composed of sesquiterpenes, lignans, neolignans, alkaloids and coumarins, has been shown to exhibit a variety of pharmacological actions (Subashini et al., 2006). *N. jatamansi* extract *(NJ)* has been reported to be a potent free radical scavenger and an antioxidant. The extract of rhizome and its ingredients provide protection in myocardial and oxidative injury (Subashini et al., 2006; Ali et al., 2000). The antioxidant effects of *NJ* in doxorubicin toxicity, was shown previously (Lyle et al., 2009).

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Accordingly, the present study aimed to investigate the possible protective effect of *NJ* against doxorubicin induced oxidative damage of the heart and liver tissues by using biochemical approaches, such as the measurement of malondialdehyde (MDA), glutathione (GSH) levels and myeloperoxidase (MPO) activity as well as by the histological analysis of tissue injury.

MATERIALS AND METHODS

Plant material and decoction preparation

Roots of *N. jatamansi* De Jones (Valerianaceae) were identified and authenticated by Dr. Sasikala Ethirajulu, (Research Officer, Botany) of the Central Institute for Siddha (CRIS), Arumbakkam, Chennai-600 101. Clean roots were air dried and powdered to prepare the alcoholic extract as earlier described (Prabhu et al., 1994). 1 kg of moderately powdered rhizomes of *Jatamansi* was extracted by refluxing with 95% ethyl alcohol in 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 out different doses for different days in the same set of rats (Subashini et al., 2006).

Test animals

Adult male Albino rats of Wistar strain weighing about 120 - 130 g were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pelleted rat chow (M/s. Hindustan Lever Ltd., Bangalore, India), 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).

Induction of experimental toxicity and treatment

The following groups of animals were used. The rats were divided into four groups (n=6 in each group): Group I: Rats served as the control; Group II: Rats were given doxorubicin (15 mg / kg body wt., intraperitoneally) on day 7 (Nagi and Mansour, 2000; Abdel-Wahab, 2003), Group III: Rats were pretreated orally with *NJ* extract (500 mg /kg body wt., orally for 7days), Group IV: Rats were pretreated orally with *NJ* extract at the above mentioned dosage for seven days and were given doxorubicin (15 mg / kg body wt., intraperitoneally) on day 7. The animals were sacrificed after 48 h of experimental period. The blood was collected and plasma separated by centrifugation at 2500 *g*. The heart and liver tissue were dissected out and washed in ice-cold saline. A portion of the heart and liver tissue were homogenized in 0.1 M Tris-HCl buffer, pH 7.4 and used for various biochemical experiments.

Analytical procedure

MPO activity was measured in tissues by a procedure similar to that documented by Vaghasiya et al., 2010, MDA formation was estimated by the method of Slater and Sawyer (1971), reduced glutathione was determined by the method of Moron et al. (1979), and plasma levels of TNF-α was determined by using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Histopathology

A small portion of the liver and heart tissue from the control and experimental animals were fixed in 10% neutral buffered formalin and processed by standard procedure for paraffin embedding and serial sections were cut (5 µM). The sections were stained with hematoxylin and eosin dyes.

Statistical analysis

The values were expressed as mean ± SD for six rats in each group. All data's were analyzed with SPSS/10 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed for inter group comparison with least significant difference (LSD) test. The values were expressed as mean ± SD, $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Doxorubicin exposure is associated with several toxic manifestations in humans and laboratory animals, in which heart, liver and kidney being the most sensitive (Mohamed et al., 2004). The results of the present study indicate that administration of DOX in a dose of 15 mg/kg, i.p. significantly elevated the cardiac and liver tissue content of MDA, and a significant decrease of GSH level. Studying the toxic effects of doxorubicin in mice previously, demonstrated that when administered chronically, doxorubicin caused multiorgan damage by increasing lipid peroxidation of the tissues (Hrelia et al., 2002). Furthermore when *Gingko biloba* extract was administered concomitantly with doxorubicin, this damage was prevented, suggesting that doxorubicin caused oxidative injury and that antioxidative agents could be beneficial against doxorubicin toxicity (Naidu et al., 2002).

As a free radical generating system, lipid peroxidation has been suggested to be closely related to oxidant-induced tissue damage, and MDA is a good indicator of the degree of lipid peroxidation (Vuchetich et al., 1996). In this study, it was observed that doxorubicin administration resulted in a significant increase in MDA levels in tissues (heart and liver) compared with the control animals.

However, these elevations were significantly reversed with *NJ* administration ($p < 0.05$) (Tables 1 and 2). The association between elevated cardiac and liver tissue content of MDA and lowered cardiac content of GSH, found in this study, strongly proves the oxidative damage caused by DOX. This observation has been supported by previous findings (Yilmaz et al., 2006; Injac et al., 2008). As shown in this study, the antioxidant *NJ* treatment significantly inhibited MDA production with a concomitant

Table 1. Effect of *NJ* on cardiac content of reduced glutathione (GSH) and melandialdhde (MDA) following doxorubicin administration

Parameter	GSH (µmol/g tissue)	MDA (nmol/g protein)
Control	4.5 ± 0.05	51.3 ± 1.55
DOX	2.8 ± 0.03*	90.0 ± 1.70*
NJ treated	4.7 ± 0.07	45.6 ± 1.04
NJ + DOX treated group	3.2 ± 0.09*	48.4 ± 1.39*

Data's are expressed as mean ± SD; n=*6*. One way ANOVA was followed by post hoc test LSD, **P < 0.05*, (Comparisons: control vs DOX induced group; DOX induced group vs *NJ* + DOX induced group).

Table 2. Effect of *NJ* on Liver tissue content of reduced glutathione (GSH) and melandialdhde (MDA) following doxorubicin administration.

Parameter	GSH (µmol/g tissue)	MDA (nmol/g protein)
Control	1.96 ± 0.12	48.36 ± 1.91
DOX	0.83 ± 0.01*	81.05 ± 3.72*
NJ treated	1.98 ± 0.18	50.61 ± 2.00
NJ + DOX treated group	1.45 ± 0.17*	60.43 ± 3.80*

Data's are expressed as mean ± SD; n=*6*. One way ANOVA was followed by post hoc test LSD, **P < 0.05*, (Comparisons: control vs DOX induced group; DOX induced group vs *NJ* + DOX induced group).

Table 3. The effects of doxorubicin and *Nardostachys jatamansi* extract *(NJ)* treatment on the myeloperoxidase activity (MPO) of the heart and liver tissues of groups.

Parameter	MPO in heart (U/g)	MPO in liver (U/g)
Control	0.62 ± 0.03	12.36 ± 1.41
DOX	6.34 ± 0.20*	28.05 ± 2.90*
NJ treated	0.60 ± 0.08	11.93 ± 1.82
NJ + DOX treated group	3.98 ± 0.71*	15.70 ± 1.87*

Data's are expressed as mean ± SD; n=*6*. One way ANOVA was followed by post hoc test LSD, **P < 0.05*, (Comparisons: control vs. DOX induced group; DOX induced group vs. *NJ* + DOX induced group).

replenishment of tissue GSH content, implying a reduction in lipid peroxidation and cellular injury, which protects the heart and liver tissues against doxorubicin-induced oxidative damage. An increase in MPO activity due to doxorubicin may cause inflammation and damage in the organs. MPO activity, which is an indicator of tissue neutrophil infiltration, was increased in all the studied tissues due to doxorubicin ($p < 0.05$) and *NJ* significantly reduced the tissue MPO activity ($p < 0.05$) (Table 3).

It has been suggested that an increase in lipid peroxidation may be due partly to the free radicals generated by neutrophils because activated neutrophils are known to induce tissue injury through the production and release of reactive oxygen metabolites and cytotoxic proteins into the extracellular fluid. When neutrophils are stimulated by various stimulants, myeloperoxidase, as well as other tissue-damaging substances, is released from the cells (Kettle and Winterbourn, 1997).

On the other hand, the proinflammatory cytokine, TNF-alpha, was found to be significantly increased, also verifying that doxorubicin toxicity is closely related with inflammatory mechanisms and oxidative damage (Figure 1). These observations have been supported by previous findings (Naiyra et al., 2010).

Since *NJ* treatment significantly decreased these cytokines and prevented the infiltration of neutrophils into the damaged tissue, the results suggest that the protective effects of *NJ* were mediated in part by blocking plasma cytokines and tissue neutrophil infiltration. This might also result in reduced lipid peroxidation and less accumulation

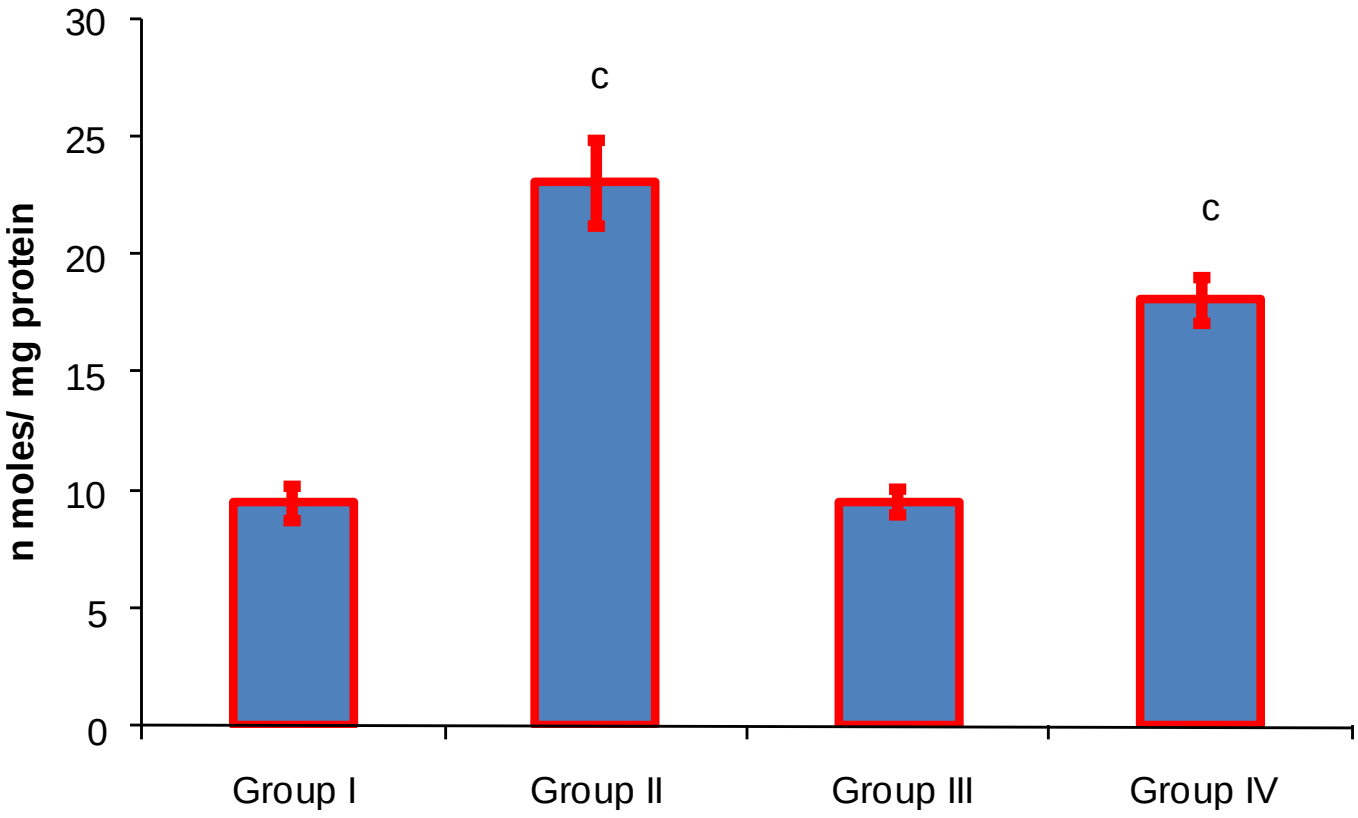

Figure 1. The effects of doxorubicin and *Nardostachys jatamansi extract (NJ)* treatment on the plasma levels of TNF-α. Group 1: Control; Group II, DOX induced; Group III, *NJ* treated; Group IV, *NJ* + DOX treated; Units: pg/ml. Values are expressed as mean ± SD; *n=6*. One way ANOVA was followed by post hoc test LSD. $^{c}P < 0.05$, (Comparisons: group I vs. group II; group II vs. group IV).

of MDA since activation of neutrophils might lead to the generation of oxygen reactive metabolites (Kettle and Winterbourn, 1997).

The histological results also verified the doxorubicin induced oxidative injury, as demonstrated by biological parameters. Figure 3 A-D shows the histological pictures of the liver cells of experimental animals. The histological examination of control rats showed normal architecture when viewed under the microscope.

The liver cells from the rats pretreated with *NJ* alone also revealed normal architecture when viewed under the microscope. Histological examination of hepatocytes from DOX treated rats showed vacuolar degenerative changes, focal necrosis, and increased kupfur cell activity, was also observed. In rats pretreated with *NJ* and administered with DOX, there was reduced overall degeneration where mild hepatocellular degeneration persisted.

Figure 2 A-D shows the histological pictures of the cardiac cells of experimental animals. The histological examination of the control rats showed normal architecture when viewed under the microscope.

The cardiac cells from the rats pretreated with *NJ* alone also revealed normal architecture when viewed under the microscope. Histological examination of cardiac cells from DOX treated rats showed myocardial coagulative necrosis, vascular dilatation and inflammatory cellular infiltration and fibrosis. Rats pretreated with *NJ* and administered with DOX, showed lesser cellular infiltration, and less myocardial necrosis than DOX-treated rats.

In the present study, increases in lipid peroxidation and myeloperoxidase activity due to the toxic effects of doxorubicin were accompanied by significant reductions in glutathione levels of the hepatic and cardiac tissues, suggesting the presence of oxidative tissue damage.

Furthermore, the elevated plasma level of the cytokine TNF-α and the histological analyses demonstrated the severity of the doxorubicin induced systemic inflammatory response. *NJ* as an antioxidant agent, amelio-rated the oxidative injury and inhibited the cytokine release.

In conclusion, the protective effects of *NJ* can be attributed, at least in part, due to its free radicals scavenging capability, ability to inhibit neutrophil infiltration and to regulate the generation of inflammatory mediators, suggesting a future role in the treatment of multiorgan damage due to drug or chemical toxicities. On the basis of our findings, it may be worthy to suggest the concomitant administration of *NJ* with DOX cancer chemotherapy.

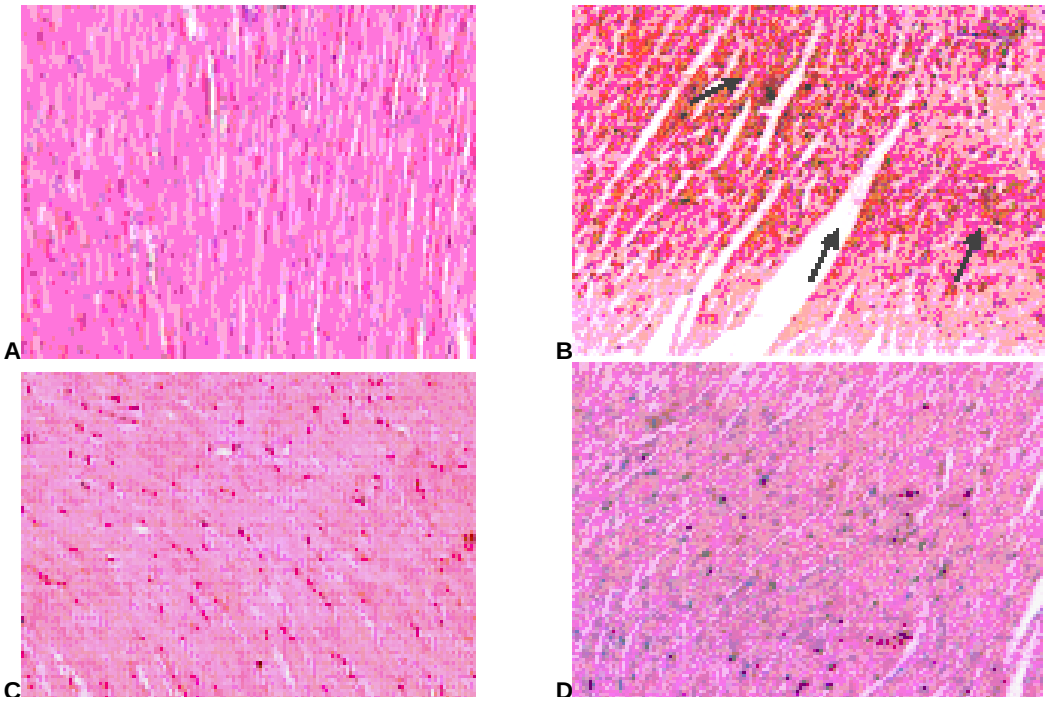

Figure 2. Histopathological changes of cardiac muscle tissue. **A.** Cardiac muscle tissue of control rats showing normal architecture H&E. **B.** Cardiac muscle tissue of DOX treated rats H&E. **C.** Cardiac muscle tissue of *NJ* treated rats showing apparently normal architecture H&E. **D.** Cardiac muscle tissue of rats treated with DOX followed by *NJ* H&E.

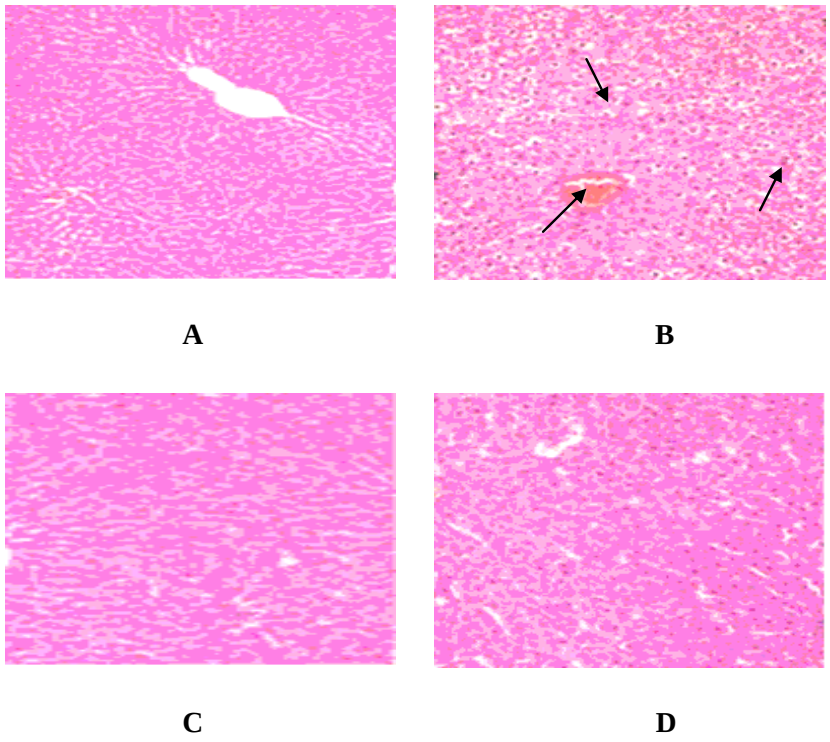

Figure 3. Histopathological changes of liver tissue. **A.** Liver of control rats showing normal architecture H&E. **B.** Liver of DOX treated rats H&E; **C.** Liver of *NJ* treated rats showing apparently normal architecture H&E. **D.** Liver of rats treated with DOX followed by *NJ* H&E.

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