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Cardioprotective effect of the root extract of *Hemidesmus indicus* against doxorubicin -induced oxidative stress in mice

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ABSTRACT

The aim of this work was to investigate possible ameliorative action of Hemidesmus indicus root extract (HiRe) against doxorubicin (Dox) induced toxicity. HiRe was administered 14 days before doxorubicin (25 mg/kg.b.wt) administration. Mouse in all groups was sacrificed 24 hrs after doxorubicin injection. Serum markers of heart injury –LDH, CPK, SGOT and SGPT levels, which were markedly increased by doxorubicin treatment, were decreased to normal levels by HiRe pre-treatment. Antioxidant enzymes-SOD, CAT and GPx, as well as GSH levels in heart tissue decreased drastically after doxorubicin injection. HiRe pretreatment elevated these levels significantly. Oxidative stress markers in heart tissue, which were high in control animals, were decreased significantly by HiRe pretreatment. The biochemical changes were consistent with histopathological observations, suggesting that Hemidesmus indicus, due to its antioxidant properties significantly reduced the oxidative stress and thereby toxicity induced by doxorubicin.

Key words: Cardiotoxicity; doxorubicin; *Hemidesmus indicus*; antioxidant.

INTRODUCTION

Although conventional method of treating cancer using chemotherapy has yielded significant clinical benefits, its full therapeutic effectiveness is masked by severe side effects. Doxorubicin (Dox) is an anthracycline antibiotic most widely used in the treatment of wide range of cancers including hematological malignancies, many types of carcinomas and soft tissue sarcomas [1, 2]. However, the clinical use is restricted due to its specific toxicities to cardiac tissues [3]. The Dox-induced cardiotoxicity has been shown to be mediated through different mechanisms, including membrane lipid peroxidation, free radical formation, mitochondrial damage and decreased activity of Na+–K+adenosine triphosphate [4].This compound inhibits topoisomerase II by intercalating DNA with high affinity and stabilizes the DNA double strand breaks Additionally, the quinine structure of anthracycline enhances the catalysisk of oxidation – reduction reactions, thereby promoting the generation of oxygen free radicals .Due to the lack of developed antioxidant defence system, these free radicals produced by electron transfer from the semiquinone to quinine moieties of the anthracycline are responsible for myocardial damage and subsequent doxorubicin induced cardiotoxicity [5].

Many studies have shown that natural products because of their safety profiles and powerful antioxidant constituents may be useful in the protection of doxorubicin induced cardiotoxicity [6, 7]. *Hemidesmus indicus Linn.* belongs to the family (Apocynaceae) [8], commonly referred to as Indian sarsaparilla, Anantamool or Nannari is a commonly available perennial climbing plant, used as the main ingredient in the preparation of the cool and refreshing drink Nannari sherbat. It is a native of India and also found in south tropical Asian countries such as Pakistan and Sri Lanka [9]. *Hemidesmus indicus* is a well known medicinal plant used for antioxidant and anti-inflammatory diseases

[10].Tribal people used this plant to treat the cancers of abdomen and skin. The root decoctions of Hemidesmus indicus R.Br. was tested on hepatoma HepG2 and EAT cells. [11,12]. The plant is used in traditional medicine in biliousness, respiratory disorders, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite and burning sensation [13,14]. The major chemical constituents are Coumarin, hemidesmine, hemidine, hemidesine and rutin [15].With all these wide spectrum of medicinal properties, the aim of our present study is to evaluate the antioxidant and cardioprotective potential of the methanolic root extract of Hemidesmus indicus on Dox-induced cardiotoxicity.

MATERIALS AND METHODS

Chemicals and reagents

Doxorubicin was obtained from Dabur Pharma Limited, New Delhi, India .Nitro blue tetrazolium (NBT), glutathione (GSH), 5'5' dithiobis (2- nitro benzoic acid) (DTNB), Thiobarbituric acid (TBA), and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits for estimating LDH, CPK, SGOT, SGPT, urea and creatinine were purchased from Agappe, Kerala, India. All other chemicals were purchased from Sisco Research Laboratories, Mumbai, India and were of highest purity grade available.

Collection of plant material and Preparation of crude extract

Hemidesmus indicus was collected from Authenticated crude drug supplier in Mysore, Karnataka, India and identification was done by a taxonomist. Identification was confirmed by depositing the voucher specimens in the Herbarium of the Department of Botany, University of Mysore. The powdered shade dried plant roots were exhaustively extracted with methanol using soxhlet extraction apparatus. The solvent of extraction was evaporated to dryness and the residue thus obtained was used for cardioprotective analysis.

Animals

8 weeks old adult male Swiss albino mice (30-32 g) were obtained from animal house, Department of Zoology, University of Mysore. Mysore, India. They were kept under standard conditions of humidity and temperature in animal house of Department of Zoology. Fed with standard mouse pellet diet, and water added libitum. Appropriate guidelines of the local animal ethics committee were followed for the animal experiments.

Experimental design

The animals were divided into 4 groups containing 6 animals each – Group I: Untreated (Normal Control), Group II: Docorubicin alone (diluted in water), Group III: Doxorubicin + HiRe (50 mg / kg b.wt) and Group IV: Doxorubicin+ HiRe (100mg /kg b.wt).

Oral administration of HiRe was started 14 days prior to doxorubicin injection. On 15th day one hour after HiRe administration doxorubicin (25 mg /kg. b.wt) was injected intra peritonially to groups II-IV. The dose of the Dox was ½ LD50. Mouse in all the groups were sacrificed after 24 hours of doxorubicin injection by ether anesthesia; blood and heart tissues were collected immediately for various biochemical analysis.

Cardiac injury enzymes

Blood samples were collected in tubes, allowed to clot and the serum was collected by centrifugation at 2000 rpm for 10 min and stored at 4 °C for analysis of various cardiac injury maker enzymes such as lactate dehydrogenase (LDH), creatine phosphokinase (CPK), serum glutamate oxaloacetate transminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) using commercially available kits.

Oxidative stress enzymes

A (10% w/v) heart tissue homogenate was prepared in ice-cold 50 mM phosphate buffer (pH 7.4),centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was used for the assays. Lipid peroxidation (LPO) in the homogenate was estimated by thiobarbituric acid method [16]. Tissue hydroperoxides and conjugated dienes were determined by the modified method of John and Steven [17].

Antioxidant enzymes

A sample of the cardiac tissue homogenate (10% w/v) was prepared by centrifugation at 10,000 rpm for 20 minutes at 4°C and was used for the analysis. Superoxide Dismutase (SOD) activity in the homogenate was measured by NBT reduction method [18]. Catalase (CAT) activity was estimated by measuring the rate of decomposition of hydrogen peroxide at 240nm [19]. Assay of glutathione peroxidase (GPx) was done based on the oxidation of GSH in the presence of H_2O_2 [20], and the level of glutathione (GSH) in the tissue homogenate was analyzed based on its reaction with 5-5' dithiobis (2-nitro benzoic acid) [21].

Histopathological analysis

A portion of heart was washed in PBS, and fixed in Bouin's fluid for 24 h and processed for paraffin embedding. Sections (4µm thick) were stained with hematoxylin and eosin and imaged with Olympus photomicroscope.

Statistical analysis

The results were expressed as mean±SD and analyzed by one way ANOVA followed by Dunnett multiple comparison test using graph pad in stat 3 software.

RESULTS

Effect of HiRe on serum marker enzymes

Levels of various marker enzymes of cardiac injury in the serum like LDH, CPK, SGOT, and SGPT were found to be drastically elevated in doxorubicin treated animals when compared to that of control animals indicating cardiotoxicity produced by doxorubicin. These increased levels were decreased significantly by HiRe pre-treatment in a dose dependent manner (Fig.1).

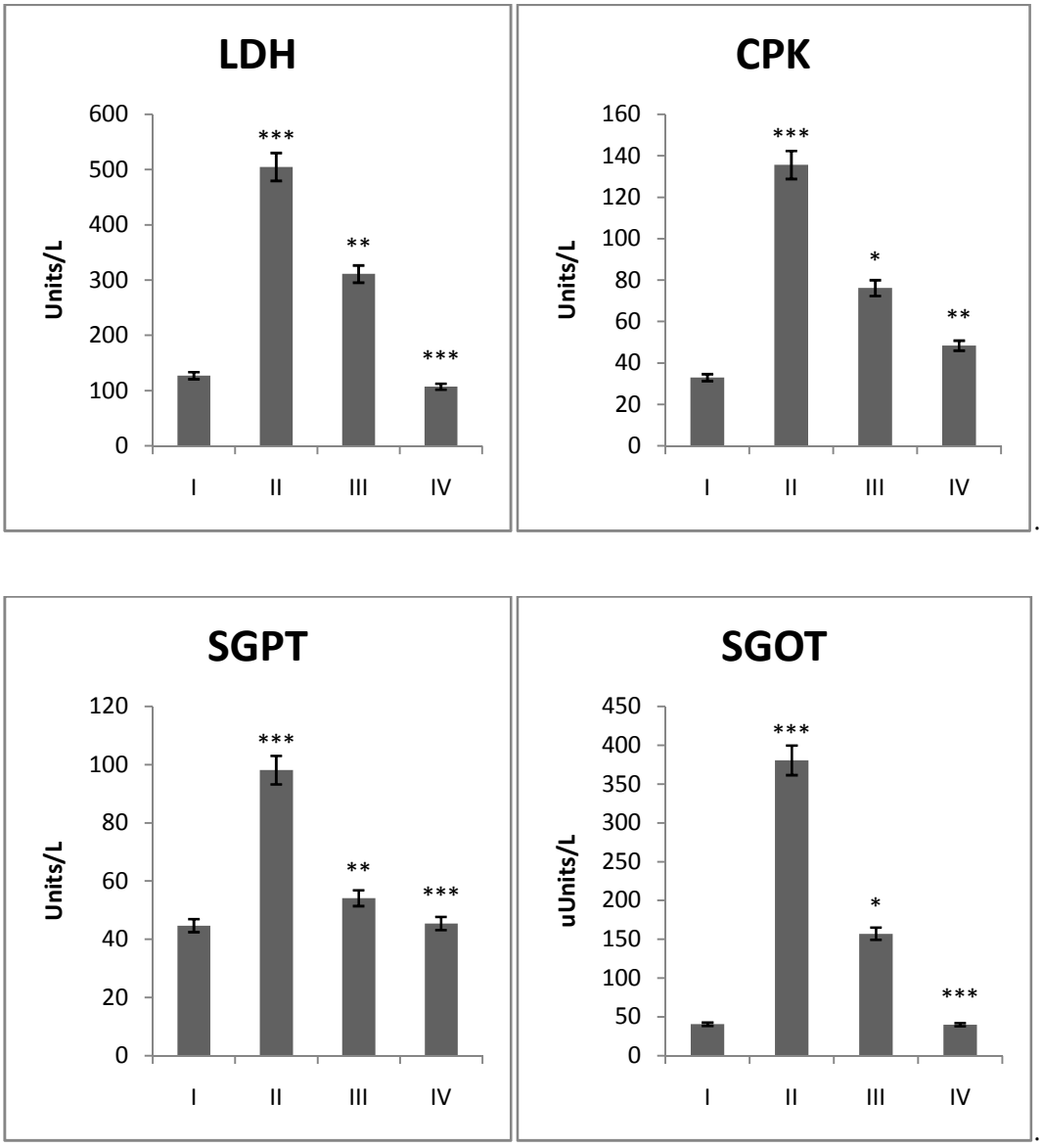


Fig.1

Protective effect of HiRe on serum enzymes during Dox induced cardiototoxicity: Group I – control; Group II –Dox; Group III – HiRe 50 mg/kg b.w. +Dox; Group IV – HiRe 100 mg/kg b.w. + Dox. LDH, lactate dehydrogenase; CPK, creatine phosphokinase; SGOT, serum glutamate oxaloacetate transminase and SGPT, serum glutamate

pyruvate transaminase.Values are expressed as mean±SD; n=6. Statistical analysis was done by using one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test.*P<0.05, *** P < 0.001, significantly against control group.

Effect of HiRe administration on antioxidant enzymes and GSH levels:

The levels of SOD, catalase, GPx and GSH in the heart tissue were decreased in the control group animals when compared to Dox treated animals. HiRe administration in a dose dependent manner inhibited this decrease and at high dose it could be restore back to normal levels (Table.1).

Table.1. Protective effect of HiRe on antioxidant enzyme levels in the heart tissue against doxorubicin induced cardiooxicity

Groups	SOD [A]	CAT [B]	GPx [A]	GSH [C]
I	**4.6±0.3**	**6.3±0.2**	**12.4±1.3**	**8.4±0.76**
II	**0.89±0.02*** **	**0.58±0.05*** **	**2.7±0.4*** **	**2.7±0.72*** **
III	**1.54±0.06[ns]**	**4.47±0.29** **	**7.26±0.67*** **	**3.4±1.12** **
IV	**2.78±0.11*** **	**7.91±0.52*** **	**9.43±0.89*** **	**9.1±0.7*** **

Treatments – I: control; II: Dox; III: HiRe(50 mg/kg) + Dox; IV: HiRe(100 mg/kg) + Dox.
*[ns] not significant,*P<0.05, *** P < 0.001, significantly (one-way ANOVA followed by Dunnet multiple comparison test) against control groups.; [A] Units/mg proteins.; [B] k/mg protein.; [C] nmoles/mg protein*

Inhibition of doxorubicin induced oxidative stress by HiRe

Formation of conjugated dienes and tissue hydro peroxides as well as LPO levels in the doxorubicin treated animals were very high compared to control group, and these parameters were reduced to normal levels in HiRe pretreated groups (Table. 2) .

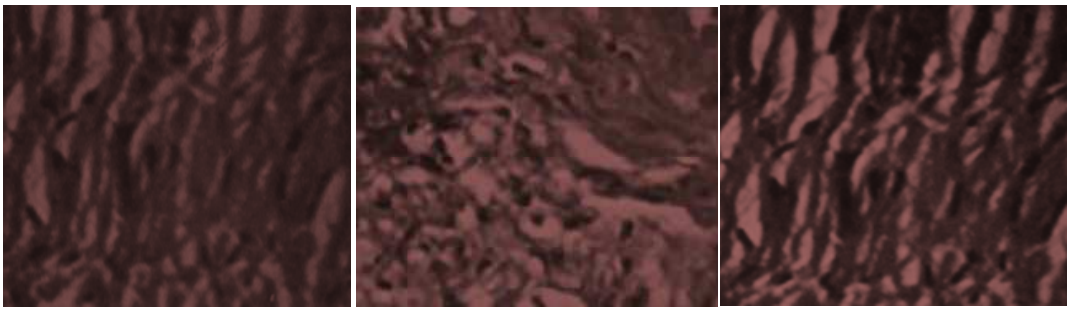
Table 2. Cardiooprotective effect of HiRe treatment on oxidative stress markers in the heart tissue against doxorubicin induced toxicity

Groups	Lipid peroxidation [A]	Conjugated diens [B]	Tissue hydroperoxides[B]
I	**3.1±0.2**	**2.4±0.12**	**6.4±0.2**
II	**6.8±0.11*** **	**5.1±0.3*** **	**8.4±0.32*** **
III	**4.1±0.2[***]**	**4.6±0.3[ns]**	**7.6±0.1** **
IV	**2.9±0.11*** **	**2.9±0.0.2*** **	**6.2±0.2*** **

Treatments – I: control; II: Dox; III: HiRe(50 mg/kg) + Dox; IV: HiRe(100 mg/kg) + Dox.
*[ns] not significant,*P<0.05, *** P < 0.001, significantly (one-way ANOVA followed by Dunnet multiple comparison test) against control groups.; [A] nmoles/mg protein.; [B] mM/100 g tissue.*

Effect of HiRe on Histopathological analysis of heart tissue:

Histopathological examination of the heart of doxorubicin administered group revealed hypertrophy of myocardium, extensive areas of haemorrhage and congested blood vessels. However pre-treatment with HiRe did not reveal degenerative signs and helped to retain the normal histology of heart (Fig. 2).



A **B** **C**

Fig.2

Cardioprotective effect of HiRe on Dox-induced heart damage. (H and E staining, magnification, x400.). A-normal histology of heart; B: Dox Treated; C-HiRe100 mg/kg b.w +Dox treated.

DISCUSSION

Current trends in cancer therapy, especially chemotherapy has made substantial progress in cancer treatment. But this modality has relatively low therapeutic index, as it is ineffective in discriminating normal tissues and neoplasia [22].The resultant broad range of organ toxicities can produce significant morbidity.

In the present study, Cardiotoxicity, which is a major side effect of doxorubicin, can be observed in control group animals as elevation in the levels of various serum marker enzymes (LDH, CPK, SGOT, and SGPT).The increased level of these enzymes indicates myocardial injury. Mild elevations of SGOT have been associated with liver injury or myocardial infarction.[23]. Higher the activity of SGOT, the larger is the injury size [24]. HiRe could significantly modulate the cardio toxicity as evident from decrease in the serum marker enzymes in the drug treated groups, which is in agreement with other studies on doxorubicin toxicities [25].

The major etiopathological factor in the doxorubicin induced cardiotoxicity was generation of free radicals, which in turn impair the antioxidant defence mechanism leading to an increased membrane lipid peroxidation, damage of membrane structure and inactivation of membrane bound enzymes [26]. Antioxidant enzymes form the first line of defence against free radicals and Cardiac tissue damage, increased oxidative stress may be due depletion of antioxidants as reported earlier [27] . In our study, Dox-treated group showed increase in LPO levels and decrease in GSH, GPx, SOD and CAT levels in heart tissue confirming the cardiac damage [28]. Depletion of GSH in mouse heart tissue due to enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption. Significant increase in the GSH, GPx, SOD and CAT activities and decrease in lipid peroxidation in heart tissue of HiRe treated groups support the above hypothesis that this increase is possibly required to overcome excessive oxidative stress caused by dox. The cardioprotective activity of HiRe was further confirmed by histopathological studies.

The antioxidant effects of Hemidesmud indicus root extract have been reported earlier [29].Antioxidant and free radical scavenger properties of HiRe possibly prevent the effects of oxidative stress [30]. This may be due to the presence of antioxidants such as flavonoids and other phenolic compounds. The antioxidants present in the HiRe have different functional properties, such as scavenging of reactive oxygen species, inhibition of generation of free radicals and chain-breaking activity. This may act as hydrogen-donating radical scavenger by scavenging lipid alkoxyl and peroxyl radical and protect the myocardium from Dox-induced injury.

CONCLUSION

In conclusion the HiRe can be considered as a good chemo protector against Dox induced cardiotoxicity, due to its non toxicity profile, acceptable route of administration (oral) and boosting the antioxidant capacity of the heart. So a combination therapy of doxorubicin with *Hemidesmus indicus* root extract will become a ray of hope for the cancer patients. Further studies on its mechanism of protection required.

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