

Protection of Doxorubicin-Induced Biochemical Injury in the Rat Bone Marrow by a Dietary Bioflavonoid Naringin

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Abstract

Doxorubicin is a secondary metabolite isolated, from *Streptomyces peucetius* and has been used as a standard chemotherapeutic agent alone or in conjunction with other chemotherapeutic drugs to treat different types of malignancies. The use of doxorubicin is associated with adverse toxic side effect on bone marrow heart, kidneys and liver in patients receiving its therapy. Therefore, any agent that can reduce the toxicity of doxorubicin will be useful in clinical conditions. Naringin a dietary flavonoid synthesized by different citrus fruits has been investigated for its protective effect against the doxorubicin-induced biochemical stress in albino rat bone marrow administered with 2 mg/kg body weight of naringin or 5 mg/kg body weight of doxorubicin either alone or in combination with each other. The bone marrow was aspirated at ½, 1 and 2 h post-doxorubicin treatment and activities of glutathione-s-transferase, catalase and superoxide dismutase, and glutathione concentration and lipid peroxidation were measured. Administration of doxorubicin alone caused a time dependent but significant reduction in the activities of catalase and superoxide dismutase and glutathione concentration followed by increased lipid peroxidation at ½, 1 and 2 h post-doxorubicin treatment. The treatment of rats with naringin before doxorubicin administration significantly raised the activities of catalase and glutathione concentration followed by reduction in the lipid peroxidation in the rat bone marrow. Our study shows that naringin attenuated the doxorubicin-induced biochemical stress and may be a useful agent in reducing the toxicity of doxorubicin in patients.

Keywords: Doxorubicin; Naringin; Rat; Bone marrow; Antioxidants

1960s from *Streptomyces peucetius* [1,2]. DOX is as one the most effective chemotherapy agents used either alone or in conjunction with other drugs or radiotherapy to treat different types of malignant neoplasia. DOX has been used to treat various malignant tumours including breast cancer, childhood solid cancers, multiple myelomas, gastric carcinoma, thyroid carcinomas, myeloblastic leukemias, ovarian cancer, small cell lung cancer, Hodgkin's and non-Hodgkin's lymphomas, Kaposi's sarcoma, neuroblastoma, osteosarcoma, nephroblastoma, Wilms tumour and soft tissue sarcomas [3-6]. The DOX has been successfully used to treat hepatic cancers [7]. The combination of DOX with lurbinedetin (PM01183) has been found to treat small cell lung cancer in phase I clinical trial [8].

The DOX acts on cancer cells during the specific stage of cell cycle, and especially the cells in the S phase of the cell cycle are highly sensitive to its effect [9]. The use of DOX in clinics is associated with the induction of numerous adverse side effects that includes alopecia, bone marrow aplasia, mouth ulcers, dizziness, myelosuppression, local aggressivity nausea, vomiting, hallucinations, vertigo, hepatotoxicity, nephrotoxicity and life-threatening cardiotoxicity, which limit its success [10,11]. DOX administration also leads to lacrimation, hypersensitivity (fever, chills, and urticaria), hyperpigmentation of the nails, and conjunctivitis [12]. Immunosuppression is another adverse effect that ensues DOX therapy making the patients more vulnerable to fatigue, microbial infections, gingival bleeding and reduced healing time [13].

The use of DOX leads to the development of life threatening cardiomyopathy both in children and adults who receive DOX for their cancer treatment [14-17]. The DOX induces cardiotoxicity in the patients employing many mechanisms. One of the predominant mechanisms of cardiotoxicity by DOX is the generation of oxygen free radicals in the cell mitochondria [14,18-20]. The DOX generates formation of reactive oxygen species (ROS), including superoxide anion radical and H₂O₂ [21,22], which in turn are produced due to redox cycling of DOX *in vivo*. The superoxide radicals undergo reaction with H₂O₂ to form highly reactive hydroxyl radicals by the iron catalyzed Haber-Weiss reaction. These secondarily derived hydroxyl radicals are highly reactive and damage proteins and DNA by triggering the onset of lipid peroxidation that stimulate a cascade of toxic reactions leading to

Introduction

Doxorubicin (Adriamycin or DOX)), a topoisomerase II targeting drug, is one of the most effective chemotherapeutic agents used in the clinic to treat cancer since its isolation in

cytotoxicity of cells and tissues [23]. The DOX intercalates into the cellular DNA and suppresses the activities of DNA and RNA polymerases leading to inhibition of nucleotide replication. It also leads to inhibition of topoisomerase II, which is crucial in the formation of DNA-cleavable complexes and the relegation of DNA during DNA replication, leading to cytotoxic effect [24,25]. Further, reductive conversion of DOX is an important mechanism of cytotoxicity and is also the main cause of drug resistance in cancer cells [26,27]. This reductive conversion of DOX is triggered by one-electron reduction of the quinone moiety of doxorubicin, by the action of NADPH and the cytochrome P450 reductase into a semiquinone radical that exerts the cytotoxic effect, or it can be oxidized back into the quinone form [28,29]. The additional oxidative stress triggered by doxorubicin may not be counteracted by endogenous system and supplementation with of exogenous antioxidants may be helpful to reduce the DOX-induced oxidative stress.

Naringin (Naringenin 7-O-neohesperidoside) is a dietary flavonoid synthesized by a variety of citrus fruits including *Citrus nobilis*, *Citrus paradise*, *Citrus junos*, *Artemisia selengensis*, *Citrus unshiu*, *Citrus sinensis*, *Artemisia stolonifera*, *Citrus tachibana*, roots of *Cudrania cochinchinensis*, aerial parts of *Thymus herba barona*, fruits of Pon cirus and other citrus species [30]. The name naringin is derived from Sanskrit 'narangi' meaning 'orange'. The grape fruit juice contains usually 800 mg/L of naringin [31]. Various preclinical studies have reported the anticancer, cardioprotective, antiviral, hepatoprotective, and neuroprotective activities of Naringin [32-36]. Naringin scavenges free radicals, chelates metals and acts as an antioxidant agent [37-45]. Naringin treatment has been found to alleviate the radiation and DOX-induced chromosome damage in earlier studies [41,46,47]. Preclinical studies in mice revealed that naringin has been able to attenuate the benzo-a-pyrene-induced forestomach carcinoma [48]. Naringin protected against the DOX-induced cardiotoxicity *in vitro* and *in vivo* [35,49]. It has been reported to be active against diabetes, inflammation, lipodystrophy, cognitive damage, osteoporosis, dyslipidemia and fibrotic disease [50-53]. Naringin reduced iron-induced toxicity *in vitro* and *in vivo* [42,54].

The cytotoxic activity of naringin has been reported against breast cancer and HeLa cells and Walker's carcinoma in rats [55-57]. The effect of naringin on the doxorubicin-induced biochemical lesions in rat bone marrow has not been studied; therefore, present study was designed to investigate the protective effect of naringin against doxorubicin-induced biochemical damage in the bone marrow of albino rats.

Materials and Methods

Chemicals

Doxorubicin hydrochloride (Biochem Pharmaceutical Industries, Mumbai, India) was procured from a local supplier, whereas naringin was purchased from Acros Organics Ltd, Geel, Belgium. Cacodylic acid, 5,5-dithio2-nitrobenzoic acid (DTNB) ethylene-diaminetetraacetic acid (EDTA), diethylene-

triaminepentaacetic acid, glutathione (reduced), nitroblue tetrazolium, phenazine methosulphate, NADH, trichloroacetic acid, and 1-chloro,2,4-dinitrobenzene were supplied by Sigma Chemicals Co, St. Louis, MO, USA. Hydrogen peroxide, potassium dihydrogen phosphate, pyrogallol, disodium hydrogen phosphate, dipotassium hydrogen phosphate, and other routine chemicals were procured from Merck India Ltd., Mumbai, India.

Animal handling

The animal care and handling were carried out in accordance with the guidelines issued by the World Health Organization, Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi, India). Generally, six to eight weeks old male albino rats weighing 45-60 g were purchased from a local source, acclimatized to laboratory conditions. The animals were kept in the polypropylene cages in the controlled conditions of temperature and light (12 h of light and dark each). The entire investigation was approved by Institutional Animal Ethics Committee (IAEC) of Mizoram University, Aizawl, India.

Preparation of drugs and mode of administration

Doxorubicin hydrochloride (DOX) and naringin (NIN) were freshly dissolved in sterile double distilled water (DDW), immediately before use. The animals were injected intraperitoneally either with 0.01 ml/g body weight (b. wt.) naringin or DOX.

Experimental

The antioxidant action of naringin was studied in the rat bone marrow, where the animals were divided into the following groups:

- Control: The animals of control group received 0.01 ml/g distilled water intraperitoneally.
- Naringin: The animals from this group were intraperitoneally administered with 2 mg/kg b. wt. of naringin [43].
- Doxorubicin: This group of animals was injected with 5 mg/kg b. wt. of doxorubicin intraperitoneally [43].
- Naringin + Doxorubicin: The animals of this group were administered with 2 mg/kg b. wt. of naringin intraperitoneally once daily consecutively for three days and one hour after the last administration of naringin, the animals of this group were intraperitoneally injected with 5 mg/kg b. wt. of doxorubicin [43].

The rats from all the groups were killed by cervical dislocation at ½, 1 and 2 hours post DOX administration. The animals were dissected and perfused with ice cold saline transcardially for the determination of glutathione (GSH), glutathione-s-transferase (GST), catalase, superoxide dismutase (SOD) and lipid peroxidation (LOO) in the rat bone marrow. A minimum of five animals were used for each dose of group in each group at each time point and a total of 60

animals were used for the whole experiment. The bone marrow cells were extracted by removing the femora of each animal at the above specified posttreatment times and placed onto a wet Whatman filter paper. The femora were freed from muscles and other tissues, cleaned and the bone marrow was extracted individually from each animal.

Preparation of homogenate

The bone marrow was weighed and 10% homogenate was prepared in phosphate buffered saline for the estimation of glutathione, glutathione-s-transferase, catalase, superoxide dismutase and lipid peroxidation.

Total proteins

The protein contents were determined using the modified method of Lowry.

Glutathione

The glutathione contents in the bone marrow of rats were determined as described earlier [58]. In brief, proteins in the homogenate were precipitated by 25% TCA, and centrifuged. The supernatant was collected transferred into another tube. It was mixed with 0.2 M sodium phosphate buffer (pH 8.0) and 0.06 mM DTNB. The whole mixture was incubated for 10 minutes at room temperature. The absorbance of the sample/s was read against the blank at 412 nm in a UV-VIS double beam spectrophotometer (Shimadzu Corporation, Tokyo, Japan) and the GSH concentration has been calculated from the standard curve.

Glutathione-S-transferase

Glutathione-S-transferase (GST) was determined as described earlier [59]. Briefly, the bone marrow homogenate was mixed with 0.1 M potassium phosphate buffer, 1 mM EDTA, glutathione reductase, and 10 mM GSH, 12 mM tert-butyl-hydroperoxide. The resultant mixture was incubated for 10 min at 37°C in water bath. The absorbance was read against the blank at 340 nm using a double beam UV-VIS spectrophotometer.

Catalase

The catalytic reduction of hydrogen peroxide was used as a measure of catalase activity by the method described earlier [60]. In brief, the samples were mixed with hydrogen peroxide and incubated at 37°C. The decomposition of hydrogen peroxide was monitored every 30 seconds by recording the absorbance against the blank at 240 nm using a UV-VIS double beam spectrophotometer.

Superoxide dismutase

Total SOD activity, was determined by the pyrogallol autooxidation method [61]. Briefly, the samples were mixed

with 62.5 mM tris-cacodylic acid buffer, containing 1 mM diethylenetriaminepentaacetic acid (DETAPA). This was followed by the addition of 4 mM pyrogallol. The autooxidation of pyrogallol was monitored against the blank at 420 nm using a UV-VIS double beam spectrophotometer.

Lipid peroxidation

Lipid peroxidation (LOO) was estimated according to the standard protocol [62]. Briefly, the samples were incubated with a mixture of trichloroacetic acid (15%), thiobarbituric acid (0.375%), and butylated hydroxytoluene (0.01%) in 0.25 N HCl at 95°C for 25 min. The reaction mixture was allowed to cool to room temperature and was centrifuged at 8,000 g. The supernatant was collected, and the absorbance was recorded against the blank using a UV-VIS double beam spectrophotometer. The lipid peroxidation has been determined against a standard curve prepared with tetraethoxypropane. For all biochemical estimations duplicate samples were used from each animal for various estimations listed above.

Statistical analysis

The significance between the treatments was determined using the Student's 't' test. A p value of <0.05 was considered statistically significant. The Solo 4 Statistical Software (BMDP Statistical Software Inc, Los Angeles, CA, USA) was used for statistical analyses.

Results

The results are shown as mean \pm standard error of the mean in **Table 1** and **Figures 1-5**.

Glutathione

The spontaneous glutathione concentration in bone marrow of non-drug treated control albino rats was estimated as 23.33 ± 2.03 nmol/mg protein. Administration of 2 mg/kg b. wt. of naringin treatment did increase the glutathione contents up to 27 ± 1.73 nmol/mg protein. However, this elevation in GSH concentration was not statistically significantly in the rat bone marrow when compared to control (**Table 1**). Doxorubicin treatment resulted in a significant decline in the GSH concentration as early as 0.5 h post treatment and this decline was approximately 2 folds lower than the control GSH concentration (**Table 1 and Figure 1**).

The GSH concentration continued to decline with assay time and almost 2.7 and 6.4-fold attrition was recorded in the GSH concentration at 1 and 2 h post-DOX treatment, respectively in the bone marrow of rats receiving 5 mg/kg b. wt. DOX alone (**Table 1 and Figure 1**). The administration of 2 mg/kg naringin before DOX treatment led to a rise in the GSH concentration in naringin+DOX treated group at all post DOX treatment times. However, a significant elevation in the GSH concentration was found only at 1 and 2 h post-DOX-treatment in naringin+DOX

treated group when compared to DOX-treatment alone (Table 1 and Figure 1).

Table 1 Effect of naringin on the doxorubicin-induced alteration in various antioxidants (Mean±standard error of the mean) at different post-DOX-treatment times in the rat Bone marrow of rats.

Treatment	Assay time (h)	nmol/mg protein				LOO nM TBARS
		Glutathione	GST	Catalase	SOD	
Control		23.33 ± 2.03	0.6 ± 0.21	10.2 ± 0.52	15.93 ± 0.66	2.33 ± 0.58
NIN		27.00 ± 1.73	0.7 ± 0.1	9.96 ± 0.24	15.73 ± 0.49	3.33 ± 0.09
DOX	0.5	12.66 ± 1.20 ^b	0.7 ± 0.11	3.73 ± 0.33 ^c	9.03 ± 0.33 ^c	9.73 ± 0.93 ^c
NIN+DOX	0.5	16.33 ± 1.20 ^a	0.66 ± 0.12	4.33 ± 0.69 ^c	9.8 ± 0.35 ^c	8.33 ± 0.97 ^b
DOX	1	8.66 ± 0.88 ^c	0.5 ± 0.05	2.57 ± 0.29 ^c	8.93 ± 0.73 ^c	11.96 ± 1.03 ^c
NIN+DOX	1	18.33 ± 0.67 ^{a*}	0.66 ± 0.09	4.83 ± 0.46 ^{c**}	9.6 ± 0.47 ^c	8.73 ± 1.24 ^b
DOX	2	3.66 ± 0.68 ^c	0.7 ± 0.12	3.03 ± 0.24 ^c	9.17 ± 0.93 ^c	16.4 ± 1.84 ^c
NIN+DOX	2	17 ± 0.53 ^{a**}	0.73 ± 0.12	5.07 ± 0.56 ^{b**}	9.73 ± 0.43 ^c	11.06 ± 0.89 ^{**}

Level of significance: *p<0.05 and **p<0.01 when Doxorubicin is compared with Naringin + doxorubicin group. ap<0.01, bp<0.001 and cp<0.0001 when Doxorubicin is compared with control group.
DOX: Doxorubicin; NIN: Naringin; GST: Glutathione-s-transferase; SOD: Superoxide Dismutase; LOO: Lipid Peroxidation.

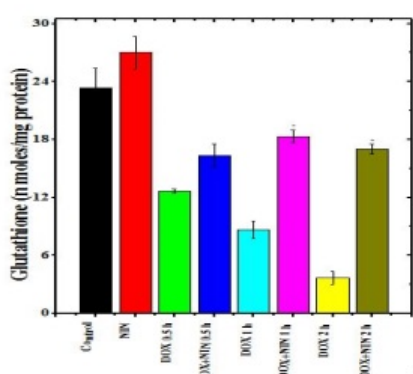


Figure 1 Alteration in the doxorubicin-induced attrition in the glutathione concentration in the rat bone marrow by naringin.

Glutathione-S-transferase

The glutathione-s-transferase activity of non-drug treated control rat bone marrow was 0.6 ± 0.21 nmol/mg/protein and naringin treatment alone did not alter the spontaneous glutathione-s-transferase activity significantly when compared to control (Table 1). Doxorubicin treatment did not lead to a significant alteration in the GST activity, at all post-DOX-treatment times (Table 1 and Figure 2). Administration of Naringin before DOX treatment marginally elevated the GST activity at all post-DOX-treatment times in the naringin+DOX group when compared to DOX treatment alone. However, this elevation was statistically non-significant (Table 1 and Figure 2).

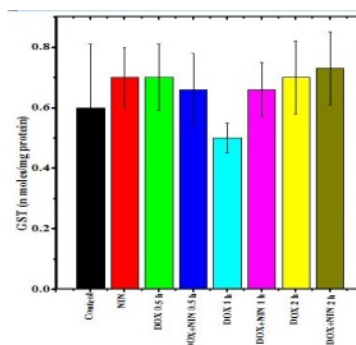


Figure 2 Alteration in the doxorubicin-induced decline in the glutathione-s-transferase activity in the bone marrow of rats by naringin.

Catalase

The spontaneous catalase activity in the control rat bone marrow was found to be 10.2 ± 0.52 nmol/mg protein and naringin pretreatment did not significantly alter the spontaneous catalase activity (Table 1 and Figure 3). The administration of DOX to rats resulted in a significant decline in the catalase activity at all post-DOX-treatment assay times when compared to control (Table 1 and Figure 3). The DOX treatment caused approximately 2.7, 4 and 3.4-fold decline in the catalase activity at ½, 1 and 2 h post-DOX-treatment as compared to spontaneous catalase activity (Table 1). Naringin treatment before doxorubicin administration increased the catalase activity, when compared to DOX treatment at all post-treatment times (Figure 3). A significant increase in the catalase activity in the bone marrow of rats was observed at all

post DOX treatment times, except ½ h where this rise was statistically non-significant (**Table 1**).

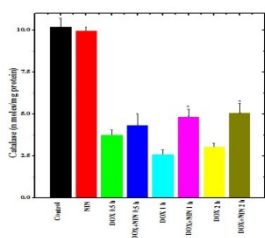


Figure 3 Alteration in the doxorubicin-induced decline in the catalase activity in the rat bone marrow by naringin.

Superoxide dismutase

The spontaneous superoxide dismutase activity in the rat bone marrow was 15.93 ± 0.66 nmol/mg protein and naringin treatment alone did not change the SOD activity significantly (**Table 1 and Figure 4**). The administration of doxorubicin alone in rats significantly reduced the SOD activity at all post-DOX-treatment assay times as compared to control (**Table 1**). The DOX treatment led to approximately 1.7-fold reduction in the SOD activity at all post-DOX-treatment times when compared to the spontaneous SOD activity (**Table 1**). The naringin pretreatment could not increase the SOD activity significantly in the naringin+ DOX group at all post DOX treatment times when compared to DOX treatment alone (**Figure 4**).

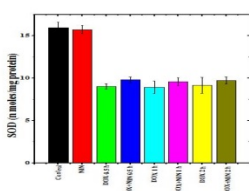


Figure 4 Alteration in the doxorubicin-induced depletion in the superoxide dismutase activity in the rat bone marrow by naringin.

Lipid peroxidation

The results of the present study show that the rate of lipid peroxidation (LOO) was 2.33 ± 0.58 nM/mg protein in the bone marrow of control albino rat. The pretreatment of rats with naringin alone did not significantly alter the spontaneous lipid peroxidation (**Table 1**). DOX treatment of rats led to a significant rise in the lipid peroxidation when compared to spontaneous levels at all assay times and a highest rise in LOO was recorded at 2 h post-DOX-treatment, where it was 16.4 ± 1.84 nM TBARS (**Table 1 and Figure 5**). The DOX treatment alone increased the LOO by 4, 6 and 8 folds when compared to control LOO (**Table 1**). Naringin treatment before DOX administration significantly reduced the rate of lipid peroxidation only at 2 h post DOX treatment when compared

to DOX treatment alone (**Figure 5**). Despite this decline, the lipid peroxidation was greater than the control group (**Figure 5 and Table 1**).

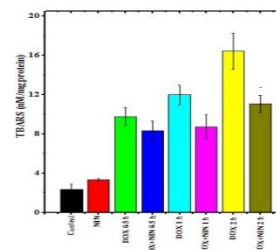


Figure 5 Alteration in the doxorubicin-induced rise in the lipid peroxidation in the bone marrow of rats by naringin.

Discussion

Doxorubicin is an important antibiotic which is in frequent clinical use to treat different cancers either alone or in combination with other chemotherapeutic drugs [6]. Bone marrow plays an important role in blood formation and immunity and it is an essential tissue in the sustenance of life. Bone marrow suppression is one of the important adverse side effects of DOX apart from cardiomyopathy and other toxic effects [10,11,63,64]. This indicates that there is a need for the pharmacological agent/s that can reduce or prevent bone marrow damage in the patients receiving DOX. Therefore, the present study was planned to study the role of naringin treatment in protecting the biochemical lesions induced by doxorubicin in the bone marrow of rats.

Gamma-glutamylcysteinylglycine or glutathione (GSH), a tripeptide thiol is ubiquitously synthesized by all eukaryotic cells, which protects cells against the oxidative stress induced by various oxygen radicals [65-67]. The antioxidant action of GSH is well proven and depletion of GSH leads to structural and functional disturbances in cells and rise in the oxidative stress [65,67]. The DOX treatment led to a time dependent attrition in the GSH concentration in the rat bone marrow which reached to a lowest level at 2 h post-DOX treatment. A similar effect has been reported earlier in the mice bone marrow [44]. These results are also in conformation with the earlier reports where DOX has been found to decrease glutathione contents in rat and mice liver and heart [35,43, 45,68]. The treatment of rats with Naringin before DOX treatment alleviated the doxorubicin depletion in the rat bone marrow is in agreement with earlier studies, where naringin has been reported to increase the GSH concentration in rat liver and mouse liver and bone marrow treated with DOX [35,43-45,68-70]. Likewise, the extract of *Aegle mermelos* has also been reported to increase the GSH contents in mice heart in an earlier study [68]. An amino acid glutamine has been reported to protect rats against DOX-induced attrition in GSH contents [71].

The enzyme glutathione-S-transferases (GSTs) are ubiquitously synthesized by all organisms and their main

function is to conjugate various endogenous and exogenous electrophilic substances with GSH. GSTs are involved in cell proliferation, and cell mortality. Apart from these functions GSTs also play an important role in detoxification and protection of DNA against various toxic insults [72,73]. The neoplastic tissues overexpress GST and it is directly linked to resistance to chemotherapy [74]. The DOX treatment did not alter the GST activity significantly in the rat bone marrow. However, earlier studies have reported a decrease in the GST activity in mice and rats [43-45]. Treatment of rats with naringin also did not affect the GST activity. In contrast, earlier studies have reported a rise in the GST activity in mouse liver, bone marrow and heart [35,44,45]. This may be due to bone marrow cell death as GSTs are involved in cell mortality.

The non-oxygen radical hydrogen peroxide has emerged as one of the major redox signalling molecule that is responsible for increased oxidative stress in the cells. It acts as a signalling molecule for cell proliferation, differentiation, inflammation and apoptosis [75]. The catalase or oxidoreductase enzymes are ubiquitously synthesized by cells and their main function is to neutralize hydrogen peroxide after its formation. These enzymes convert H_2O_2 into water and molecular oxygen [76,77]. Their reduced activity increases accumulation of H_2O_2 and oxidative stress. The DOX administration reduced the activity of catalase enzyme in the bone marrow of rats, which was lowest at 2 h post-DOX treatment. In earlier studies DOX treatment has been found to reduce the catalase activity in mouse and rat heart, liver and bone marrow [35,43-45,68,71]. Catalase activity showed an increase in the rat bone marrow treated with naringin before DOX administration is in agreement with earlier studies, where a similar effect has been observed in the liver and hearts of rats and mice liver and bone marrow cells [35,43-45, 69,70]. Likewise, the extract of *Aegle marmelos* has been found to protect mice heart against the DOX-induced depletion in the catalase activity [68].

Superoxide dismutase are redox metalloenzymes (SOD), which are produced by all organism that use oxygen for their energy requirements. The SODs are located inside the cell as well in the extracellular matrix [78,79]. They provide protection against the superoxide anion produced during respiration by converting it into less harmful product H_2O_2 . The SODs degrade H_2O_2 into harmless products like water and molecular oxygen [78,79]. DOX administration has led to a time dependent depletion in the SOD activity in the bone marrow cells of rat and lowest activity was recorded at 2 h post-DOX treatment in the present study. The cardiotoxic, hepatotoxic and nephrotoxic action of DOX is mediated through its interaction with eNOS that in turn elevates the superoxide radical generation [80]. This may have resulted in the of reduced SOD activity in our study. The DOX treatment has been reported to alleviate SOD activity in mice bone marrow, liver and heart earlier [35,44,45,68]. DOX treatment has also decreased the SOD activity in the rat liver [43]. Naringin treatment marginally alleviated the DOX-induced decline in rat bone marrow however the increase in SOD activity was not statistically significant. Naringin treatment has been found to decrease the DOX-induced attrition in the SOD activity in rat liver and heart and mice bone marrow, liver and

heart in earlier studies [35,43-45,69,70]. Likewise, mice heart receiving extract of *Aegle marmelos* before DOX administration showed elevation in the SOD activity [68].

Lipids in the form of triglycerides are used to store energy and they are also integral part of cell membrane [81]. The lipids are targeted by free radicals and H_2O_2 via Fenton reaction and produce lipid peroxides, which are index of cytotoxicity [82]. DOX induces ROS, which produce lipid peroxides to trigger cancer cell killing [28]. This may be the reason for time dependent increase in the lipid peroxidation in the rat bone marrow cells treated with DOX. These findings are in conformation with earlier studies where DOX treatment has been found to increase lipid peroxidation [35,43-45,68-70,83]. Naringin treatment reduced the DOX-induced lipid peroxidation in rat bone marrow is in agreement with the earlier studies, where a similar effect has been reported [35,43-45,69,70]. Naringin has been reported to protect against the bleomycin-induced lipid peroxidation in rat lung [52]. Likewise, L-glutamine has been also reduced the DOX-induced lipid peroxidation in rat heart in an earlier study [71].

The exact mechanism of protection of DOX-induced biochemical lesions by naringin in rat bone marrow is not known. It is speculated that naringin may have used different mechanisms to reduce DOX-induced toxicity. The scavenging/inhibition of DOX-induced free radicals by naringin may be one of the important mechanisms of action, which would have reduced the burden on GSH and other enzymes resulting in their increase in the bone marrow when compared to DOX alone treatment. DOX-utilizes NADPH oxidase system to trigger free radical formation and the presence of naringin has been reported to inhibit the activation of NADPH oxidase, which may have arrested the free radical formation [84,85]. The presence of iron increases the DOX-induced free radical formation and iron chelating action of naringin would have blocked this action, which would have led to an increase in the antioxidants in the rat bone marrow in the present study [35]. Naringin has been reported to upregulate the gene expression of catalase, and SOD that may have resulted in the elevated antioxidant status in the present study [40]. The nuclear factor erythroid 2-related factor 2 (Nrf2) is constitutively expressed by all tissues and DOX has been reported to inhibit its expression causing depletion in the endogenous antioxidants. The presence of naringin may have reduced this ability of DOX leading to a rise in the antioxidant status when compared to DOX alone. Naringin has been reported to upregulate Nrf2 [86].

Conclusions

DOX treatment caused an attrition in the endogenous antioxidants in the rat bone marrow which may due to its ability to produce free radical-induced oxidative stress. The naringin pretreatment may have inhibited the DOX-induced free radical formation leading to elevated antioxidant status in the rat bone marrow. This increase in endogenous antioxidants by naringin may be due to the inhibition of NADH oxidase and upregulation of Nrf2 elements in the rat bone marrow.

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Conflict of Interest Statement

Authors do not have any conflict of interest statement to declare.

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