Research Article

Terminalia muelleri Attenuates the Accumulation of Excess Iron, Inhibition of Topoisomerase 2ß and Oxidative Cardiac Damage in Doxorubicin-Induced Cardiotoxicity in Rats

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Abstract | Background: This work aims to evaluate the protective and attenuating effect of *Terminalia Muelleri* ethanol extract (TME) against doxorubicin-induced cardiac toxicity. Methods: Total phenolic content (TPC), total flavonoid content (TFC), DPPH· radical scavenging, FRAP and FRAC were determined in the TME extract. Forty-eight adult male Wistar rats were divided equally into six groups: control non-treated, Doxorubicin (DOX) challenge (rats were injected with 2.5 mg/kg of DOX in six injections over 2 weeks), TME was solely administered for four weeks, two weeks pre and two weeks co-administration of TME (100 mg/kg) with DOX as well as TME (100 mg/kg) and TME (200 mg /kg) were administered after two weeks of DOX injection. Results: A relatively high TPC and TFC with considerable antioxidant capacity as evaluated by DPPH, FRAP, and FRAC examinations were recorded. DOX significantly increased serum AST, LDH, CK-MB activities, Troponin1, iron accumulation, and oxidative stress as evidenced by the increased MDA, and NO in association with significant reduction of GSH content in cardiac tissue. Furthermore, mRNA expression of iNOS was significantly upregulated and eNOS and Top 2 ß mRNA expression levels were downregulated. The pre and concomitant administration of TME with DOX as well as the post-administration of TME at 100 and 200 mg /kg suppress the harmful effect of DOX on heart tissue. Conclusion: TME is a potent cardiac protective agent that protects and preserves the heart tissue against the deleterious effect of doxorubicin.

Keywords | *Terminalia Muelleri; Doxorubicin;* iNOS; eNOS; Top 2 ß

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INTRODUCTION

Cardiovascular system dysfunction is one of the most frequent consequences of exposure to antineoplastic treatment, whereas cardiovascular diseases and cancers are the leading causes of morbidity and mortality in the world (Sung et al., 2021). The less regeneration ability of cardiac cells renders cardiac myocytes to be more prone to the long-term adverse effects of chemotherapy agents such as anthracycline (Hardaway, 2019).

Cardiotoxicity is the toxicity that affects the heart. This definition is attributed to the effect of chemotherapy on the entire cardiovascular system that occurs through di-

rect damage to the heart muscle or disturbances in cardiac electrophysiology or cellular pathways, resulting in either cardiac failure or cardiac arrest (Cardinale et al., 2020). The prevalence of cardiovascular disorders among chemotherapy-treated patients could represent a risk of health problems than the development of cancer recurrence (Beyer et al., 2019).

Doxorubicin (DOX, the trade name Adriamycin) is a cytotoxic agent that comes under the class of anthracycline antibiotics (Frederick et al., 1990), and is one of the pillars of cancer treatment (Alexieva et al., 2014). DOX treatment in patients shows both effects like therapeutic and toxic effects. It has been reported that the cardiomyopathy initiated by doxorubicin is a progressive and multifactorial process (Wu et al., 2022), including oxidative stress, iron accumulation with the alteration of gene and protein expression and DNA breakage via inhibition of topoisomerase II (Kong et al., 2022).

The management of cardiac injury before and during cancer treatment is essential to reduce morbidity and mortality in cancer patients. Dexrazoxane is a cardio-protective agent for anthracycline-induced cardiotoxicity. It provides cardiac protection through metal-chelating activity with the prevention of reactive oxygen species. It also acts as a catalytic inhibitor of DNA topoisomerase II (Thougaard et al., 2010). It has been reported that dexrazoxane interferes with some mechanisms of anthracyclines with the potentiation of cardiotoxicity (Lipshultz and Herman, 2018). Beta-blockers (BBs) such as Carvedilol, block ß 1, ß 2, and α1- adrenergic receptors and have potent antioxidant and anti-apoptotic properties, which justified their use for primary prevention of anthracycline cardiotoxicity (Kalay et al., 2006). The cardiotoxic preventing effects of beta blockers depend mainly on which specific receptor subtype is being blocked, it has been suggested that ß2- adrenergic receptors play a cardio-protective role in the pathogenesis of cardiomyopathy, meanwhile, it was found that the ß1- adrenergic receptors subtypes mediated some of the acute anthracycline cardiotoxicity. Due to their efficacy in the treatment of ailments, medicinal plants have long been sources of traditional and even modern medicines (Hussien et al., 2022; Ali et al., 2022). These plants are rich sources of phytochemical substances that have a clear physiological function in the human body system (Karamoka et al., 2019). It has been observed that a wide range of substances present in foods and plants exhibit several physiological properties, such as antioxidant and anticancer activity in the treatment of heart failure (Mantawy et al., 2014; Gu et al., 2015; Ma et al., 2016).

The *Combretaceae* family contains the genus *Terminalia* Linne, which includes *Terminalia muelleri* (TM). Due to its exceptional phytochemistry and strong antioxidant content, *Terminalia muelleri* has received a lot of attention (Bernstein et al., 2005). Numerous types of active components, including tannins, pentacyclic triterpenes and their glycoside derivatives, flavonoids, and other phenolic compounds, were found in phytochemical research on Terminalia muelleri (Fahmy et al., 2015; Rashed and Barreto, 2017; Elmalah et al., 2022). A major obstacle to be taken into account when choosing a cardio-protective drug to combat DOX-induced cardiotoxicity is the concurrent assessment of this agent's anticancer activity (Wenningmann et al., 2019). Elsenosi et al. (2019) supported the antiproliferative mechanisms of *T. muelleri* phytochemicals in the prevention of tumorigenesis in rats caused by diethyl nitrosamine (DENA) revealing a significant decrease in the immune-histochemical staining expression of Proliferating cell nuclear antigen (PCNA) following administration of *T. muelleri* extract. Additionally, Elmalah et al. (2022) noted that the mitigation of inflammation, oxidative stress, and extracellular matrix proteolysis in hepatocellular carcinoma induced in rats was made by *Terminalia mulleri* leave extract that could be a more valuable as an *a*nticancer adjuvant agent. Since doxorubicin causes cardiac oxidative damage, we hypothesize that *Terminalia muelleri* ethanol extract (TME) may offer a protective and relieving activity that could be a promising therapeutic potential. To achieve this, the current study was conducted to determine the total phenolic and flavonoid content of TME. This was done in conjunction with an investigation into the antioxidant activity of TME and an assessment of its protective and curative effects against doxorubicin-induced cardiotoxicity.

MATERIALS AND METHODS

Preparation of *Terminalia muelleri* ethanolic extract (TME)

The leaves were dried in the shade and ground into powder. After that, the powder was placed within a soxhlet apparatus and continuously percolated using ethanol (95% v/v) as the solvent. Under a vacuum evaporator, the extract was concentrated (Pandya et al., 2013). The extract was kept in a vacuum desiccator at 4 °C and shielded from light and humidity until use. It was a solid, dark green extract. To generate a final concentration of 10 mg/ml of TME for use in some phytochemical experiments, 500 mg of TME extract was dissolved in 5 ml of methanol and then completed to 50 ml with double distilled water.

Some phytochemical studies on the *Terminalia muelleri* extract

Determination of Total phenolic and total flavonoids contents: The total phenolic content of the extract was determined using Folin-Ciocalteau (Singleton et al., 1999). The standard gallic acid curve was prepared and the total

phenol values were expressed in the term of mg Gallic acid equivalent/g of plant material. The total flavonoid content was determined according to the method by Meda et al. (2005). The standard quercetin curve was prepared and total the flavonoid content was expressed as quercetin (QU) equivalents (mg /g extract).

Evaluation of the antioxidant activity of TME extract: 2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging assay was performed according to the method of Molyneux (2004), Ferric reducing antioxidant power (FRAP) assay was carried out according to the method of Al-Farsi et al. (2005) and Ferrous reducing antioxidant capacity (FRAC) of TME extract was estimated by the method of Oyaizu, (1986).

Doxorubicin (DOX)

Doxorubicin hydrochloride vials were obtained from Eimic Company, Cairo, Egypt. DOX was prepared and diluted under sterile conditions in 0.9% saline to obtain a dose of 2.5mg/ kg b. wt. of rats according to Ma et al. (2016).

The experimental design

Forty-eight adult male Wistar rats, weighing 150 – 200 g were obtained from the animal house, Research Institute of Ophthalmology, Giza, Egypt. All rats were maintained in an air-conditioned animal house with specific pathogen-free conditions and were subjected to a 12:12-h daylight/darkness cycle. The animals were allowed free access to water and rats were fed on the standard diet that was a mixture composed of 72.2% carbohydrate, 3.4% fats, 19.8% proteins, 3.6% cellulose, 0.5% vitamins, and minerals as well as 0.5% salts. The standard diet was obtained from Kahira Company, Cairo, Egypt. Rats were kept at constant environmental and nutritional conditions through the period of acclimation (one week) and the experimental period. Animal usage and care for experimental protocol were approved via the Institutional Animal Care and Use Committee (IACUC) of NODCAR No. II/49/19.

The experimental animals were divided into five experimental groups beside a control group (8 rats of each) as the following; Group 1: Normal control, group 2: DOX challenged rats: DOX was IP injected (2.5mg/kg b. wt.) with two days intervals to get a total dose of 15 mg/kg body weight/week. group 3: rats were administered a single dose of TME (100 mg/kg b. wt) orally for one month (Mabrouk et al., 2022), group 4: rat of this group received a daily oral dose (100 mg/kg b. wt.) of TME for two weeks then IP injected with DOX in concomitant with the daily oral administration of TME for two weeks, group 5: rats were IP injected with DOX (2.5mg/kg b. wt.) for two alternative days/week for two weeks followed by a daily oral dose (100 mg/kg b.wt.) of TME for two other weeks and group 6: rats were IP injected with DOX (2.5mg/kg b. wt.) for two alternative days per week for 2 weeks followed by a daily oral dose (200 mg/kg b. wt.) of TME for two weeks. Blood samples from the retro-orbital vein were taken at the end of the experiment, and sera were separated. Fresh hearts were immediately removed, and each one was divided into two parts. One part was mixed with phosphate-buffered saline to prepare the cardiac tissue homogenate for the measurement of the contents of cardiac ferritin, reduced glutathione (GSH), lipid per-oxidative end product malondialdehyde (MDA), and nitric oxide (NO). The second was kept in RNA cell lysis for Real-Time Polymerase Chain Reaction (RT-PCR) analysis for Inducible Nitric Oxide Synthase (iNOS), Endothelial Nitric Oxide Synthase (eNOS), and Topoisomerase 2beta (Top2ß).

The evaluated parameters in Serum and Cardiac tissue

Measurement of the serum cardiac enzymes and cTn I levels: Serum activities of AST, LDH, and CK-MB were determined according to the methods of Reitman and Frankel (1957), Vassault et al. (1983), and Wu and Browers (1982). Serum levels of Troponin I was measured by Dean's (1998) method.

Determination of the serum total iron and cardiac Ferritin levels: Serum iron was determined according to the method of Conrad and Umbreit (2000) and Cardiac Ferritin content in heart tissue homogenate of rats was determined using kits provided by Novus Biologicals NBP2-67953, (Rats FE, Bio-techne brand, USA). The determinations were performed according to the manufacturer's instructions.

Determination of MDA, GSH contents, and NO level in cardiac tissue: The measurements of MDA, GSH, and NO were performed according to the methods of Ohkawa et al. (1979), Beutler et al. (1963), and Miranda et al. (2001). Cardiac protein concentration was calculated by using the standard curve of bovine serum albumin (BSA) solution.

Quantitative real-time PCR for the mRNA expression levels of iNOS, eNOS, and Top2 ß in cardiac tissue: Using a Qiagen tissue extraction kit and following the manufacturer's instructions, total RNA was extracted from cardiac tissue. For the cDNA, conversion cDNA reverse transcription kit, total RNA (0.5-2 g) was used (Fermentas, USA). Using RNA sequences from the Gene Bank, PCR primers were created using the Gene Runner program (Hasting Software, Inc., Hasting, NY). The calculated annealing temperature for each set of primer was 60 °C. In a 25 ml reaction volume, quantitative RT-PCR was carried out using 2x SYBR Green PCR Master Mix

(Applied Biosystems), 900 nM of each primer, and 2-3 l of cDNA. Two minutes at 50 °C, ten minutes at 95 °C and forty cycles of denaturation for 15 s and annealing/extension for ten minutes at 60 °C were used for the amplification process (Abdelghany et al., 2022). The Applied Biosystems Software was used to calculate data from real-time tests (Foster City, CA). The comparative threshold cycle approach was used to calculate the relative expression of the mRNAs for iNOS, eNOS, and Top2ß (Livak and Schmittgen, 2001). The assay was carried out three times, and the 2CT method was used to analyze the relative gene expression data. The ß -actin gene served as the standard for all values.

Table 1: Sequence of the primers used for real-time PCR.

Gene	Primer sequence
eNOS	F 5′-CGA GAT ATC TTC AGT CCC AAG C-3′ R 5′-GTG GAT TTG CTG CTC TCT AGG-3′
iNOS	F5′-CACCACCCTCCTTGTTCAAC-3′ R 5′-CAATCCACAACTCGCTCCAA-3′
Top 2ß	F-5′- ATTGTGGCCCTCATGACCAG -3′ R5′- TTGAGAGCGACCCCAGTTTC -3′
β-actin	F 5′-GAGAAGATCTGGCATCACAC-3′ R5′-ATCAGGTAGTCTGTCAGGTC- 3′

Statistical analysis

Data are expressed as mean ± standard error (S.E). The Kolmogorov-Smirnov test was employed to verify the normal distribution, and the variables were found to be normally distributed. Differences between groups were assessed by one-way analysis of variance (ANOVA). Subsequent multiple comparisons between the different groups were analyzed by Duncan's multiple comparison test. A correlation study was performed to estimate the relation between the quantitative variables by Pearson correlation (r). Data were statistically analyzed using the statistical package of Social science (SPSS) version 23. The graphs were drawn using a prism computer program (GraphPad Software Inc. V5, San Diego, CA, USA). Values at $p < 0.05$ were considered significant (Jatinder and Anil, 2017).

RESULTS

Results of the phytochemical studies

The amount of Total phenolic (TPC) and flavonoid (TFC) contents in TME: The obtained results revealed that TME extract contains a measurable amount of phenolic compounds concerning Gallic acid, approximately 343±11.92 mg GAE/g extract, and a high amount of flavonoid content, approximately 488.33±8.57 mg Quercetin /g extract.

Results of the antioxidant activity of TME extract

DPPH radical % scavenging activity of TME extract: Table 2 reveals that the DPPH radical % scavenging activity of TME increased with the increment of the extract concentration. Table 2 shows the IC_{50} values which denote the concentration of TME extract required to scavenge 50% of DPPH radical.

Table 2: The DPHH radical % scavenging activity of TME extract

Concentration µg /ml	% Scavenging of TME extract
6.25	25 ± 1.1
12.5	40 ± 1.6
25	53 ± 1.5
50	79.6 ± 1.4
IC50	24.04 ± 0.13 µg /ml

Values are mean of 3 determinations for each conc. ± SD

Ferric reducing antioxidant power (FRAP): The obtained data revealed that the extract elicits a ferric-reducing power equivalent to 210.78 ± 6.94 µM Fe (II)/100 g extract. This value reflects the redox properties of the polyphenol contents of the extract that play an important role in adsorbing and neutralizing free radicals.

Reducing power of TME extract: The reducing power of TME is shown in Figure 1. The reducing power of the extract indicates that the extract has a reduction potential that reduces potassium ferricyanide (Fe^{+3}) to potassium ferrocyanide (Fe^{+2}), and the reducing power increases as the concentration of the examined extract increase.

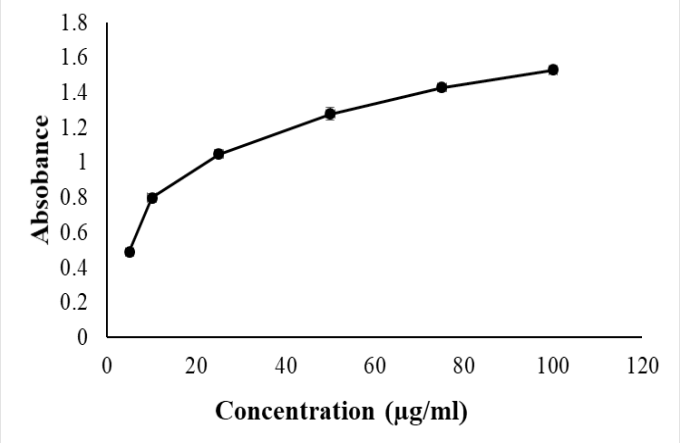

Figure 1: Reducing power of TME extract

Results of the present study reveal that the sole administration of TME extract to rats for four weeks exerts no change in all the examined parameters (Table 3 and Figs 2-9).

Table 3: Serum AST, LDH, CKMB activities and cTn I level in the control group, DOX-injected rats and under the influence of different treatments of TME extract

Parameters/Groups	AST (U/L)	LDH (U/L)	CKMB (U/L)	cTn I (ng/ml)
Control	21.8 ± 1.6[a]	64.7 ± 1.1[a]	116.3 ± 2.70[a]	0.60 ± 0.02[a]
DOX	66.3 ± 4.1[e]	145.2±4.2[e]	246.8 ± 15.1[e]	2.3± 0.12[d]
TEM	19.5 ± 1.5[a]	63.9 ±1.3[a]	112.4 ± 4.7[a]	0.59 ± 0.03[a]
TEM +DOX+TEM	36.5 ± 1.6[c]	90.4 ± 2.1[c]	160.4 ± 3.4[c]	0.8 ± 0.04[b]
DOX+TEM100	44.7 ± 2.5[d]	110.4 ± 2.2[d]	185.5 ± 5.4[d]	1.01 ± 0.04[c]
DOX+TEM 200	29.7 ± 1.7[b]	76.4 ± 1.5[b]	144.7 ± 3.3[b]	0.71 ± 0.05[ab]

Data are expressed as mean values ± S.E. (n = 6 rats). In the same column; values with different superscript letters are significantly differ ($p < 0.05$).

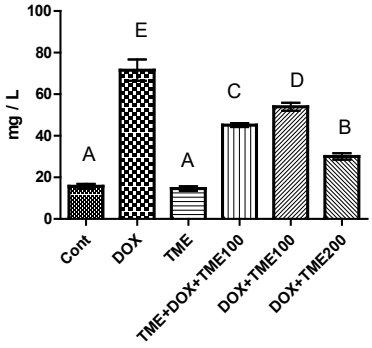

Figure 2: Serum iron (mg/L) of rats in control group and under the influence of different treatments. Each bar represents a mean value ±SE and the presence of different letter on the bar means a significant difference between groups

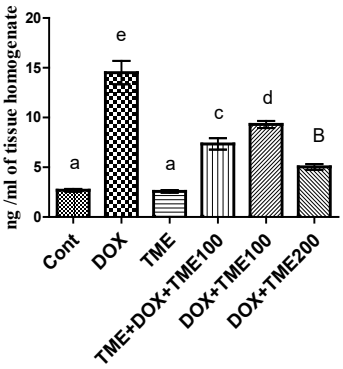

Figure 3: Serum iron (mg/L) of rats in control group and under the influence of different treatments. Each bar represents a mean value ±SE and the presence of different letter on the bar means a significant difference between groups

DOX ADMINISTRATION INDUCED CARDIAC TOXICITY

In the present study, administration of DOX in an accumulative dose (2.5mg/kg b. wt.) every other day (total of six injections) caused a significant release of cardiac enzymes (AST, LDH, and CK-MB), Troponin I (cTnI) and total iron in the circulation. DOX also induced significant increases in the level of Ferritin, MDA, and NO in cardiac tissue in association with a significant reduction in cardiac GSH content. DOX injection up-regulates the mRNA expression of iNOS in association with down-regulation of the mRNA expression levels of eNOS and TOP 2ß. Table 2 shows the correlation between the examined parameters under the influence of DOX injection. The obtained results revealed a strong relationship between the accumulation of total iron, induction of oxidative stress in the heart tissue, the up-regulation of iNOS, down regulations of eNOS, and TOP 2ß in the induction of cardiac toxicity as evidenced by the evaluation of serum cardiac marker enzymes and cTnI.

TME alleviates the enzymatic activity of serum AST, LDH, CKMB, and Troponin I (cTn I) level: Data presented in Table 3 reveal that DOX-injected rats elicited a significant increase in the activities of the examined enzymes and cTn1 when compared with the control non-treated group. Table 3 also shows that the two weeks and concomitant with DOX injection reduced the induced increase in the activity of the examined enzymes and Troponin I concerning the DOX injected group. The administration of TME at a dose of 100 and 200 mg /kg b.wt/day for two weeks to DOX-challenged rats significantly reduces the induced increments in the examined enzymes and cTn1 when compared to DOX-challenged rats, and TME at higher dose exhibits a more decreasing effect on the examined enzymes and Troponin I (c Tn I).

TME extract decreases serum total iron and cardiac Ferritin levels in DOX- challenged rats: Figures 2 and 3 demonstrate that the IP injection of DOX for two weeks in an accumulative dose of 15 mg/Kg induced a significant increase ($p < 0.05$) in the serum level of total iron compared to the control non-treated group in association with a significant increase in cardiac Ferritin level. Figs 2 and 3 also show that the two weeks pre and two weeks co-administration of TME with DOX significantly attenuated ($p < 0.05$) the induced increase in serum total iron and cardiac Ferritin levels compared to DOX-injected rats. The

obtained data also reveal that the two weeks post administration of TME either in low or high doses to DOX-challenged rats reduced the induced increase in serum iron and cardiac Ferritin levels compared to DOX-injected rats. The obtained data also reveal that TME at high doses exerted a more pronounced reducing effect on total serum iron and cardiac Ferritin.

TME extract improves the Redox status of Cardiac tissue

TME extract attenuates the generation of free radicals in Cardiac tissue: Data in Fig. 4 prove that one of the most causes of heart injury in normal tissue is the use of DOX which leads to the production of free radicals as evaluated by the obtained significant increase of cardiac MDA level in DOX injected rats when statistically compared with the control non-treated group. The obtained results also reveal that the pre and co-administration of TME at a low dose (100 mg/kg b. wt.) with DOX exerted a significant protective effect against the generation of free radicals as manifested by the obtained decrease in cardiac MDA level in comparison DOX- injected rats. The administration of TME extract either in low or high doses two weeks after DOX injection exerted a significant decreasing effect on cardiac MDA and the more pronounced decreasing effect was displayed by the high dose of TME administration (200 mg/kg) as shown in Fig. 4.

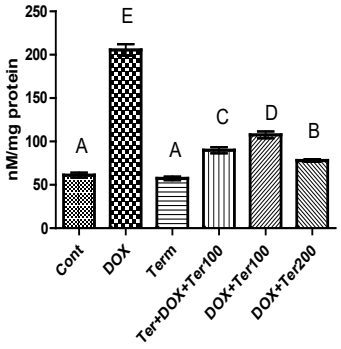

Figure 4: MDA in Cardiac tissue (nM/mg protein) of rats in control group and under influence of different treatments. . Each bar represents a mean value ±SE and the presence of different letter on the bar means a significant difference between groups

TME extract increases GSH contents in Cardiac tissue: Fig. 5 shows a significant reduction in cardiac GSH of challenged rats. The gained results reveal that the two weeks of pre and co-administration of TME at a low dose (100 mg /Kg) with DOX caused a significant increment in cardiac GSH content when compared with DOX-challenged rats (Fig. 5). Administration of TME extract at low or high doses for two weeks significantly increased GSH content in the cardiac tissue of DOX injected rats. The obtained data also reveals that however, TME in high doses displayed a more pronounced effect in increasing GSH, the pre and co-administration of TME with low doses exerted a protective role that could be near to that obtained by the administration of TME at high doses.

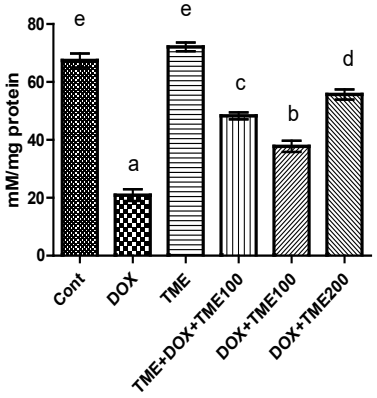

Figure 5: GSH in Cardiac tissue (mM/mg protein)) of rats in control group and under influence of different treatments. . Each bar represents a mean value ±SE and the presence of different letter on the bar means a significant difference between groups

Effect of TME on the production of NO, mRNA expression levels of iNOS and eNOS in Cardiac tissue

TME extract decreases the production of NO in DOX-injected rats: Injection of DOX in an accumulative dose (15 mg/Kg) induced a significant elevation in NO content of cardiac tissue compared to the control non-treated group (Fig.6). This increment in NO level is significantly attenuated by the two weeks pre and co-administration of TME with DOX. The administration of TME either in low or high doses significantly decreased the cardiac NO level. The recorded data reveal that the treatment of DOX-injected rats with a high dose of TME exerted a more pronounced effect in decreasing the production of NO (Fig.6).

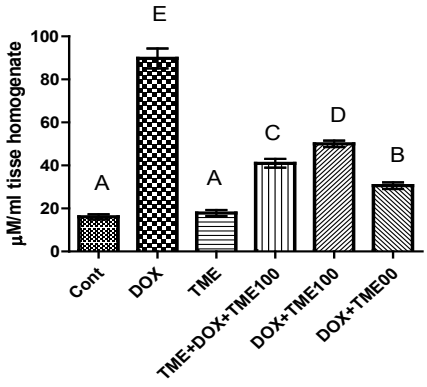

Figure 6: NO content in Cardiac tissue (µM/mg protein)) of rats in control group and under influence of different treatments. . Each bar represents a mean value ±SE and the

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presence of different letter on the bar means a significant difference between groups

TME decreases the mRNA expression level of iNOS in DOX-injected rats: The obtained data reveal that DOX injection significantly up-regulates the mRNA expression level of iNOS in cardiac tissue concerning the control non-treated group. This increase is significantly alleviated with the two weeks pre and concomitant administration of TME relative to DOX-injected rats. The obtained data also reveal that the treatment of DOX-challenged rats with TME either in low or high doses significantly attenuated the expression level of iNOS compared to DOX-injected rats and the most significant decreasing effect is obtained in the group of rats treated with the high dose of TME (Fig.7).

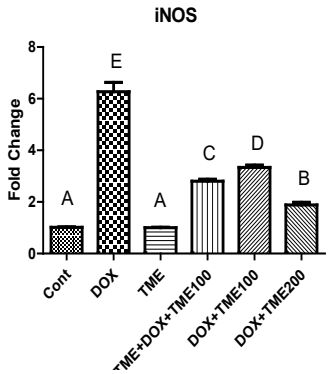

Figure 7: The mRNA expression level of iNOS in Cardiac tissue of rats in control group and under influence of different treatments. The steady-state level of Each bar represents a mean value ±SE and the presence of different letter on the bar means a significant difference between groups of mRNA in the heart were analyzed by RT-PCR assay. Beta actin was used as an invariant internal control for calculating mRNA fold changes. Each bar represents mean value ±SE (n=6). Mean values with different superscript letters are signinifcantly different.

TME improves the mRNA expression level of eNOS in DOX-injected rats: Figure 8 reveals that the oral administration of TME extract for four weeks exerts no effect on the expression level of eNOS mRNA in cardiac tissue compared to the control non-treated group. Meanwhile, the IP injection of DOX to normal r at caused a significant decrease in the mRNA expression level of eNOS in cardiac tissue compared to the control non-treated group. This decrement is significantly abrogated by the pre and co-administration of TME with DOX (Fig.8). Treatment of DOX cDOX-challenged with TME either in low or high doses significantly improved the expression level of eNOS in the heart tissue. A more pronounced increasing effect on the mRNA expression level of eNOS is recorded in the group of rats treated with high a dose of TME administration (Fig. 8).

TME extract protects TOP 2ß in cardiac tissue: The inhibitory effect of DOX in an accumulative dose on TOP 2ß is manifested in the current by the obtained down-regulation in mRNA the expression level of TOP 2ß compared to the control-non-treated rats (Fig.9). The two weeks pre and co-administration of TME with DOX significantly increased mRNA expression level of TOP 2ß when compared with DOX- injected rats (Fig.9). The obtained data also reveal that the treatment of DOX injected rats with TME either in lower or higher dose significantly improved the mRNA expression of TOP 2ß, and the treatment of DOX challenged rats with a high dose of TME exerted a more pronounced improvement against the deleterious effect of DOX on the expression level of TOP 2ß (Fig.9).

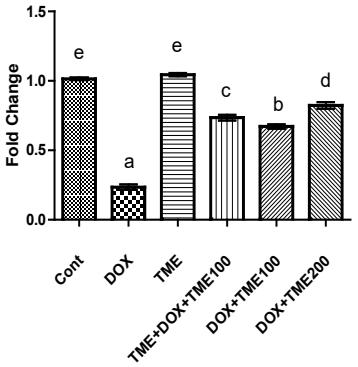

Figure 8: The mRNA expression level of eNOS in Cardiac tissue of rats in control group and under influence of different treatments. The steady-state level of Each bar represents a mean value ±SE and the presence of different letter on the bar means a significant difference between groups of mRNA in the heart were analyzed by RT-PCR assay. Beta actin was used as an invariant internal control for calculating mRNA fold changes. Each bar represents mean value ±SE (n=6). Mean values with different superscript letters are signinifcantly different.

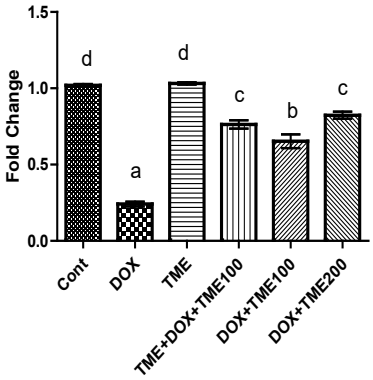

Figure 9: The mRNA expression level of Topoisomerase 2β in Cardiac tissue of rats in control group and under

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influence of different treatments. The steady-state level of Each bar represents a mean value ±SE and the presence of different letter on the bar means a significant difference between groups of mRNA in the heart were analyzed by RT-PCR assay. Beta actin was used as an invariant internal control for calculating mRNA fold changes. Each bar represents mean value ±SE (n=6). Mean values with different superscript letters are signinifcantly different

DISCUSSION

Our study evaluated *Terminalia mulleri's* potential for cardiac protection as obtained by the considerable amount of high levels of total phenolic and flavonoid contents (de la Rosa et al., 2018). Flavonoids are a family of phenolic chemicals that are widely present in fruits and vegetables and greatly contribute to their antioxidant action. They are distinguished by a benzo-pyrone structure (Khalaf et al., 2019). These findings agree with those of Mabrouk (2013), who used phytochemical analysis to screen TM and found that it contained phenolic and relatively high flavonoid contents including kaempferol, quercetin, apigenin, luteolin coumarins, terpenes, apiol, monoterpenes, myristicin, and furanocoumarins. Flavonoids have been suggested as chemopreventive agents or dietary supplements due to their diverse spectrum of biochemical and physiological effects.

Myocardial-specific enzymes like AST, LDH, and CK-MB, as well as serum Troponin I, are considered to be important measurements for determining the extent of the cardiac injury during a myocardial injury to investigate the potential therapeutic and preventive effects of *Terminalia muelleri* ethanol extract (TME) against DOX-induced cardiotoxicity (Ananthan and Lyon, 2020). This study explains the higher cardiovascular risk associated with DOX treatment by attributing the observed increase in AST, LDH, and CK-MB activity in the circulation to their release from the injured cells. The increased cardiovascular risk linked to greater levels of these enzymes in the blood may be explained by the release of cardiac enzymes from the myocardium under situations of stress, acute coronary syndromes, or reperfusion-related injury.

This study explains the higher cardiovascular risk associated with DOX treatment by attributing the observed increase in AST, LDH, and CK-MB activity in the circulation to their release from the injured cells. It is said that the increased cardiovascular risk linked to greater levels of these enzymes in the blood may be explained by the release of cardiac enzymes from the myocardium under situations of heightened stress, acute coronary syndromes, or reperfusion-related injury (Danese and Montagnana 2016; Ndrepepa 2021).

Since cardiac Troponin I (cTnI) is only released during myocardial necrosis, it is thought to be a highly sensitive and specific marker of myocardial cell injury. Serum levels of cTnI are closely correlated with the severity of myocardial injury (Lakhani et al., 2021). In the current study, elevated serum cardiac enzymes and Troponin I represent a loss of cell membrane integrity that makes myocardial cells more porous and permeable or may rupture, allowing cTnI to escape into the bloodstream. According to this research, it is crucial to look for Troponin I in cancer patients receiving chemotherapy, particularly anthracycline, to identify cardiotoxicity.

In the current work, rats exposed to DOX-induced a large rise in serum iron levels along with a similar considerable rise in heart tissue ferritin levels. This result supports the findings by Gulati et al. (2014) that high iron accumulation contributes to cardiotoxicity, which leads to heart failure in DOX users. It was reported that DOX can change iron metabolism through its greatest affinity to this metal-forming Iron-DOX complexes that inactivate both Iron-regulatory proteins 1 and 2 (IRP1and IRP2) with perturbation the iron-response elements (IREs) of iron metabolism to act as either a translational enhancer or inhibitor in cardiomyocytes (Christidi and Brunham, 2021). The interaction of DOX with the iron-responsive elements (IREs) of the ferritin heavy and light chains is what led to the observed rise in cardiac ferritin levels in the current investigation. However, ferritin functions as an iron transporter, reducing free iron inside cells; consequently, when this protein becomes disorganized, there is an increase in free iron, which damages the myocardium (Vela, 2020).

A unique, controlled cell death pathway called ferroptosis can be brought on by specifically targeted lipid peroxidation. Ferroptosis is characterized by the buildup of lipid hydroperoxides to deadly quantities and is dependent on ferrous iron (Rawat et al., 2021; Kong et al., 2022). The results of the current investigation showed a substantial positive association between MDA (lipid per-oxidative end product) production and the elevated level of blood total iron, which suggests that ferroptosis was induced in DOX-challenged rats. This result is consistent with that of Tadokoro et al. (2020), who showed that ferroptosis is perceived as a substantial kind of cell death in cardiomyocytes in DOX-induced cardiotoxicity.

A significant contributor to the etiology of DOX-induced heart dysfunction is thought to be lipid peroxidation. Once DOX penetrates the cell, it produces excessive ROS and extremely cytotoxic free radicals, which causes cardiac membrane function and integrity to be lost (Octavia et al., 2012; Sun et al., 2013; Shabalala et al., 2017). The release of AST, LDH, CKMB, and cTn I indicated a substantial

association between the level of oxidative stress and the severity of tissue damage. This discovery supported DOX's involvement in the creation of heart cell injury. According to reports, one of the mechanisms of DOX-induced cardiac toxicity is oxidative stress, and oxidative stress-mediated ROS plays a significant role in DOX cardiac toxicity (Gorini et al., 2018).

The cardiac toxicity induced by DOX is mediated by excess iron buildup, enhanced lipid peroxidation, and GSH depletion. Therefore, it has been claimed that preventing heart cell damage is a useful cardioprotective tactic (Whelan et al., 2010). The total flavonoids included in the ethanol extract of *Terminalia muelleri* are significant. Flavonoids have been suggested as chemopreventive agents or dietary supplements due to their diverse spectrum of biochemical and physiological effects. Their ability to chelate iron, act as antioxidants, and scavenge free radicals are regarded to be the main biological mechanisms at work (Scalbert et al., 2005). According to the results of the current investigation, the pre and concurrent administration of TME with DOX considerably reduced the serum iron level and the amount of cardiac ferritin. This finding proved the efficacy of flavonoid compounds in TME extract in ameliorating iron status by reducing the saturation of iron such as ferritin. The same findings were also obtained through the administration of TME either at low or high doses to DOX-challenged rats and support the previous studies that reported the free radical scavenging capacity of flavonoids were obtained after forming complexes with metal ions (Wang et al., 2021).

The fact that TM is a polyphenol-rich herb means that its polyphenol compounds will be able to directly bind reactive oxygen species and scavenge them or act as sacrificial antioxidants to block the lipid peroxidation cascade, as was the case in the current study. The results of the current study also showed that DOX-challenged rats were treated with TME at low and high dosages and that both levels dramatically reduced lipid peroxidation and mitigated cellular membrane damage.

The mRNA expression level of eNOS was significantly reduced in the current study's DOX-exposure rats. This finding is consistent with that of Akolkar et al. (2017) and He et al. (2020), who found that DOX toxicity might prevent the phosphorylation of eNOS and that endothelial nitric oxide synthase (eNOS) expression was considerably downregulated. According to reports, DOX affects the formation of the monomer/dimer ratio of eNOS by influencing oxidative stress or oxidation cofactor, which causes eNOS uncoupling, which is linked to several pathological conditions and prevents it from producing the physiological levels of eNOS-derived NO necessary for cardiovascular functions (He et al., 2020).

Data from the current study elicited that, pre and co-administration of TME with DOX, and administration of TME either at low or high doses attenuated the production of NO level, iNOS mRNA expression, and increase eNOS mRNA expression levels. This finding strongly proves the role of iNOS-produced NO in myocardium dysfunction and supports the protective role of eNOS against DOX-induced myocardial injury.

The enzyme DNA topoisomerase II (TOP II) is essential for repairing DNA topology (Nabhan et al., 2015). The isoenzymes Top2α and Top2β express topoisomerase 2 (Top2) (Wang, 2021). The capacity of doxorubicin to form ternary complexes with homodimeric TOP2 subunits and DNA, leading to DNA double-strand breaks and DNA damage in tumor cells, was reported to be the cause of its tumor-curing effects (Chihara et al., 2016). Topoisomerase IIB (Top 2ß), on the other hand, is more prevalent in dormant cells, such as adult mammalian cardiomyocytes, and its expression is stable throughout the cell cycle. Doxorubicin forms the Top2-DOX-DNA complex in cardiac cells, which causes DNA double-strand breaks and activates the DNA damage response and apoptosis. (Armstrong et al., 2016). Cardiac Top2 ß mRNA expression was significantly decreased by DOX. This result demonstrated the genotoxic nature of DOX and its ability to cause cardiac damage in normal cells (Shi et al., 2018). Data from the current study showed a very high positive correlation between the levels of eNOS and Top2 expression down-regulation, and their magnitudes of down-regulation are very similar, indicating that the disruption of nitrogen homeostasis may be a major factor in decreasing the expression of Top2; however, this finding needs to be further explored in a future study.

It is important to note that DOX significantly decreased topoisomerase 2 through DNA intercalation; this mechanism is also responsible for DOX's toxicity and the death of cancer cells. It has been reported that the deletion of the Top2 gene prevented the heart from suffering damage, proving that this interaction partially mediates cardiotoxicity (Zhang et al., 2012). Dexrazoxane, a medication that lowers Top2- levels, also protected the heart from cardiac harm (Kalyanaraman, 2020). Given the importance of topoisomerase to cell survival, the contradictions here are noteworthy. Through the preservation of Top2 expression, pre- and co-administration of TME with DOX demonstrated a strong protective role in the current study against the unintended effects of DOX. Two weeks after the DOX challenge, the expression level of Top2 was considerably raised by the injection of low or high dosages of TME. These results lend credence to the phytochemical TM's

protective and attenuating effects against Top 2ß's interaction with DOX to cause cardiac toxicity. This conclusion agree with that made by Modesto et al. (2021), who claimed that green tea phytochemicals protect Top2 from the cardiotoxicity caused by the association with DOX.

CONCLUSION

The result of the present study demonstrates that TME at 100 and 200 mg /kg suppress the harmful effect of DOX on heart tissue through the chelation of excess iron, inhibition of lipid peroxidation and protection against glutathione depletion. TME also suppresses the production of reactive nitrogen species and protects the Top 2ß against the inhibitory effect of DOX with the improvement of cardiac function. In conclusion, TME could be used prophylactically as a possible adjuvant therapy to reduce the adverse effects of Doxorubicin on the cardiac tissue of cancer patients.

RECOMMENDATION

We recommend the administration of TME to patients treated by doxorubicin to ameliorate its injurious effects.

ABBREVIATIONS

DOX	Doxrubicin
DPPH	2, 2-diphenyl-1-picrylhydrazyl
eNOS	endothelial nitric oxide synthase
GSH	reduced glutathione
iNOS	Inducible nitric oxide synthase
MDA	Malondialdehyde
NO	nitric oxide
RT-PCR	Real-Time Polymerase Chain Reaction
TFC	total flavonoid content
TME	*Terminalia Muelleri* ethanol extract
Top2ß	Topoisomerase 2beta
TPC	Total phenolic content

DATA AVAILABILITY

All data will be available on reasonable request.

CONFLICT OF INTEREST

The authors declare that they don't have any conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the study including design of the experiments, methodologies, analysis and interpretation of results and drafting the manuscript.

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