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Cardioprotective Effect of Tanopati against Doxorubicin-induced Myocardial Toxicity in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AKN carried out the cardioprotective tests and drafted the manuscript. Author KK performed the histopathological analysis. Author KKS helped for the interpretation of the histopathological results. Author YHF made the statistical analysis. Author DAJ participated in the design of the study and helped to draft the manuscript. Author NJD conceived the study, participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of this study was to determine the preventive role of Tanopati against doxorubicin induced myocardial toxicity in rats.
Study Design: Randomized experimental controlled study.
Place and Duration of Study: This study was carried out in Laboratory of Biochemical Pharmacodynamy, Félix Houphouët-Boigny University of Abidjan, Côte d'Ivoire between July 2014 and January 2015.

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Methodology: Twenty five albino Wistar rats, divided into five groups with five rats each, were used in this study. Cardiotoxicity was induced by doxorubicin(dox) (15 mg/kg for 2 weeks). Tanopati (10 mg/kg orally) or vitamin E (100 mg/kg orally) was administered as pretreatment for two weeks, and followed by dox for another two weeks. Biomarkers like lactate dehydrogenase (LDH), creatine phosphokinase (CK), iso enzyme CKMB, lipid peroxidation activity, antioxidants such as glutathione (GSH), and antioxidants enzymes such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels were monitored 36 hours after administration of the final dose. Histopathological examination was performed.
Results: The repeated administration of dox (2.5 mg/kg of body weight) caused cardiomyopathy associated with an antioxidant deficit. Pretreatment with Tanopati decreased LDH (417.6±1.17 to 324.6±1.7 UI/L), CK (328.2±0.8 to 230.5±1.09 UI/L) and CKMB (234.9±1.03 to 172.2±2.06 UI/L) levels compared to the values in the control group. Tanopati significantly protected the myocardium from the toxic effect of dox, by increasing the levels of antioxidants such as GSH (1.56±0.03 to 1.76±0.02 nmol /g of heart tissue), SOD (21.66±0.34 to 29.93±0.13 U/g of protein), and CAT (40.13±0.65 to 46.57±0.55 µmol H_2O_2/min/mg of protein) and decreasing the level of malondialdehyde (MDA) (48.09±1.0.83 to 25.46±0.7 nmol/g). Tanopati also reduced the severity of cellular damage of the myocardium as observed microscopically.
Conclusion: The results obtained suggest that cardioprotective effect of Tanopati might be attributed to its antioxidant activity.

Keywords: Tanopati; antioxidant; cardiotoxicity; doxorubicin; free radicals.

ABBREVIATIONS

BW: body weight; ANOVA: analysis of variance; dox: doxorubicin; TBARs: thiobarbituric acid reactive substances; SEM: standard error of means; CK: Creatine kinase; LDH: lactate dehydrogenase; CAT: catalase; MDA: malondialdehyde; SOD: superoxide dismutase.

1. INTRODUCTION

Doxorubicin (dox) is one of the most effective antitumor antibiotics belonging to the class of anthracyclines. However, its use is limited by a high incidence of irreversible myocardial damage and dilatation [1].

Congestive heart failure, cardiomyopathy and electrocardiographic changes were demonstrated after cumulative dox administration [2]. Excessive formation of free radicals and the high level of oxidative stress produced by the anthracyclines have been suggested to play important role in promoting oxidative myocardial damage [3]. In this regard, therapeutic interventions having antioxidant effect may offer considerable cardioprotection [4]. Dox-induced cardiotoxicity may thus serve as an appropriate model to study the effect of oxidative stress on the cardiac tissues and the therapeutic efficacy of drugs even in cancer-free laboratory animals [5].

Several approaches may be taken to decrease the risk of dox-induced cardiotoxicity while maintaining its efficacy. These include altered schedules of drug administration, modifications of the anthracycline molecule, adjunctive treatment with beta-adrenergic blockers, angiotensin-converting enzyme inhibitors (ACEi), dexrazoxane, and probucol [6,7]. None of these have been entirely successful. A new drug to prevent or treat Dox-induced cardiotoxicity is therefore needed.

The therapeutic actions of most medicinal plants are related to their antioxidant properties which, in turn, could be ascribed to their antioxidant phytochemicals [8]. The cardioprotective effect of various medicinal plants and plant products have been documented [9-11]. Sustainable agents from natural sources could serve as viable alternatives to currently available synthetic drugs in the management of cardiovascular-related disorders. This is especially important owing to the toxic side effects of most synthetic drugs and their high costs which make them not readily accessible to many patients in developing countries like Cote d'Ivoire.

Our earlier study has demonstrated that Tanopati a polyherbal formulation is not toxic (LD50>2000 mg/kg BW) and rich in antioxidant compounds [12]. The dose used was 10 mg/kg daily estimated from the information given by traditional practitioners.
The aim of the present study was to investigate the possible effects of Tanopati against dox-

induced cardiotoxicity in rats using biochemical markers and histopathological analyses.

2. MATERIALS AND METHODS

2.1 Animals

Male Wistar rats weighing 150-200 g were procured from the Animal House of the Faculty of Pharmaceutical and Biological Sciences Félix Houphouët-Boigny University of Abidjan. Animals were housed in plastic cages where they had free access to water and food, and kept at temperature of 22±3ºC with a relative humidity of 50.15%. The cycle of light and darkness was 12 h/12 h. All the experimental procedures were approved by the Ethical Committee of Health Sciences, Félix Houphouët-Boigny University of Abidjan. These guidelines were in accordance with the European Council Legislation 87/607/EEC for the protection of experimental animals.

2.2 Plant Material

Tanopati is a recipe obtained from the decoction of roots, leaves and bark of plants used in the Ivorian traditional medicine. The plants include *Ageratum conyzoides, Newbouldia Laevis, Phyllanthus muellerianus, Aloe vera* and *Cassia occidentalis*.

2.3 Chemicals and Drugs

Doxorubicin and vitamin E were obtained from local pharmacy, Abidjan, Cote d'Ivoire, Tanopati was gifted by an Ivorian traditional practitioner, glutathione, thiobarbituric acid (TBA), 5′, 5′-dithiobis- 2-nitrobenzoic acid (DTNB), and trichloracétic acid (TCA) were from Merck Co. (Germany). All reagents, solvents and chemical compounds used for analysis met the quality criteria in accordance with international standards.

2.4 Preparation of Lyophilized Extract of Tanopati

This recipe was provided by M. Adou Tano Albert, an Ivorian traditional practitioner.

2.5 Experimental Protocol

After one week of acclimatization, the animals were randomly divided into five groups of five animals in each.

Group 1: Normal saline 5 ml/kg body weight (i.p.) as control.

Group 2: Animals were treated with dox (2.5 mg/kg body weight, i.p.) in 6 equal injections alternatively for two weeks to make a total cumulative dose of 15 mg/kg body weight.

Group 3: Animals received Tanopati (10 mg/kg body weight po) for two weeks and then alternatively with vehicle (normal saline) for next two weeks.

Group 4: Animals received Tanopati (10 mg/kg body weight for two weeks) as a pretreatment followed by dox (2.5 mg/kg body weight, i.p.) administration as in group 2.

Group 5: Animals received vitamin E (100 mg/kg body weight for two weeks) as a pretreatment followed by dox (2.5 mg/kg body weight, i.p.) administration as in group 2.

2.6 Enzyme Assays

Thirty six hours after the last treatment, orbital blood samples were obtained under light ether anesthesia using serum separating tubes for the estimation of isoenzyme CKMB and LDH. CK, CK-MB and LDH activities were determined according to standard methods using diagnostic kits cobas Integra 400 Plus Roche/SIGMA. Animals were sacrificed under ether anesthesia and a midline abdominal incision was performed. Cardiac tissue was quickly dissected out and washed in ice cold saline, dried on filter paper and weighed immediately. A portion of each heart was taken from all the groups and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of GSH [13], MDA [14], CAT [15], and SOD [16].

2.7 Histopathological Parameters

Heart tissue sections were fixed in 10% formalin. The specimens were processed by standard procedure and embedded in paraffin wax. The blocks were sectioned from the ventricular portion, stained of hematoxylin and eosin and examined by light microscopy (x 100 magnification).

2.8 Statistical Analysis

The results are expressed as mean ± S.E.M. The results were analyzed using one-way ANOVA [17] followed by Turkey's multiple comparison tests. Data was computed for statistical analysis

by using Graph Pad Prism 5 Software. P values < 0, 05 were considered as significant [18].

3. RESULTS

The effect of Tanopati on dox-induced cardiac toxicity was established by measuring cardiac biomarker enzymes, endogenous antioxidants and by examining cardiac tissue microscopically

3.1 Heart Weight, Body Weight and Ratio of Heart Weight to Body Weight

The Effects of dox on heart weight, body weight and ratio of heart weight to body weight are shown in Table 1. The heart weight and ratio of heart weight to body weight in dox-treated group are significantly increased compared to normal group. The heart weight and ratio of heart weight to body weight in Tanopati+dox and vitamin E + dox treated group were significantly less compared to the group receiving only dox.

3.2 Serum Enzyme Biomarkers

Animals treated with dox showed significant increase in the levels of CK, CKMB and LDH compared to normal group (Table 2). Tanopati+dox and vitamin E + dox treated group showed significant lower levels of CK CKMB and LDH compared to dox treated group.

3.3 Antioxidant Status

The effects of dox on tissue lipid peroxidation, antioxidant and antioxidant enzymes are shown in Table 3. The MDA levels were increased. GSH, SOD and CAT levels were significantly decreased in Dox-treated group compared to normal group. Tanopati+ dox and vitamin E+ dox treated group showed significant decrease (P<0.05) in the level of MDA and increase in the status of GSH and antioxidant enzymes.

3.4 Histopathological Parameters

Histopathological images of heart are shown in Figs. 1 to 5.

Control and Tanopati (10 mg/kg) group rats showed normal cardiac fibers without any damage (Figs. 1 and 2). The heart sections obtained from dox-treated animals showed abundant areas of necrosis and aggregation of acute inflammatory cells and damaged vascular spaces (Fig. 3). Animals pretreated with Tanopati 10 mg/kg (Fig. 4) and vitamin E 100 mg/kg (Fig. 5) showed improvement in the cell integrity evidenced by absence of necrosis, less vacuolization of the cytoplasm and maintenance of normal integrity of the cardiac muscles.

Table 1. Effect of Tanopati on body weight, heart weight and heart/body ratio in dox-treated rats

Treatment group	BW(g)	HW (g)	HW / BW ratio ($\times 10^{-3}$)
G1: Normal saline 5 ml/kg BW	195.5±0.81^{c}	0.6±0.01^{a}	3.05±0.02^{a}
G2: 2.5 mg/kg BW of dox	158.2±4.1^{a}	0.72±0.01^{c}	4.55±0.08^{c}
G3: 10 mg/kg BW of Tanopati	197.5±1.31^{c}	0.58±0.01^{a}	2.91±0.03^{a}
G4: 10 mg/kg BW of Tanopati +dox	184.5±1.56^{b}	0.66±0.01^{b}	3.55±0.05^{b}
G 5: 100 mg/kg de BW of VitE+dox	177.1±1.72^{b}	0.67±0.01^{b}	3.76±0.04^{b}

Values are means±SEM for 5 rats. ^{a,b,c} Row values with different superscripts are significantly different (p< 0, 05). BW: body weight, HW: heart weight, VitE: Vitamin E, dox: doxorubicin.

Table 2. Effect of Tanopati on CK, CKMB and LDH enzyme activities in dox-treated rats

Treatment group	C K (UI/L)	LDH (UI/L)	CKMB (UI/L)
G1: Normal saline 5 ml/kg BW	154.4±1.56^{a}	238.5±0.81^{a}	133.7±1.4^{a}
G2: 2.5 mg/kg BW of dox	328.2±0.8^{c}	417.6±1.17^{d}	234.9±1.03^{d}
G3: 10 mg/kg BW of Tanopati	154.8± 1^{a}	256.7±1.52^{b}	144.2±2.47^{b}
G4: 10 mg/kg BW of Tanopati +dox	230.5±1.09^{b}	324.6±1.7^{c}	172.2±2.06^{c}
G5: 100 mg/kg BW of vitE+dox	227.4 ±1.21^{b}	320.5±1.46^{c}	170.6±2.45^{c}

**Values are means±SEM for 5 rats. ^{a,b,c} Row values with different superscripts are significantly different (p< 0, 05). BW: body weight, LD: Lactate Dehydrogenase, CK: creatinekinase, VitE: Vitamin E, dox: doxorubicin, GSH: Glutathion*

Table 3. Effect of Tanopati on oxidative status in dox-treated rats

Treatment group	MDA (nmol/g heart tissue)	GSH (nmol/g of tissue)	CAT (µmolH_2O_2/min /mg of protein)	SOD (U/mg of protein)
G1: Normal saline 5 ml/kg BW)	16.93±0.69[a]	2.89±0.07[c]	59.9±0.41[d]	36.9±0.66[d]
G2: 2.5 mg/kg BW de dox	48.09±0.83[c]	1.56±0.03[a]	40.13±0.65a	21.66±0.34[a]
G3: 10 mg/kg BW of Tanopati	18.59±1.38[a]	2.78±0.05c	59.73±0.98[d]	38.44±0.88[d]
G4: 10 mg/kg BW of Tanopati+dox	25.46±0.7[b]	1.76±0.02[b]	46.57±0.55[c]	29.93±0.13[c]
G5:100 mg/kg BW of vitE+dox	23.94±0.33[b]	1.67±0.02[a]	43.14±0.14[b]	27.61±0.37[b]

Values are means ± SEM for 5 rats. [a,b,c] Row values with different superscripts are significantly different (p< 0, 05). BW: body weight (p< 0,05). BW: body weight, VitE: Vitamine E dox: doxorubicin, MDA: Malondialdehyde, SOD: Superoxide Dismutase, CAT: catalase, GSH: Glutathion, U: one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of nitrobluetetrazolium (NTB) reduction.

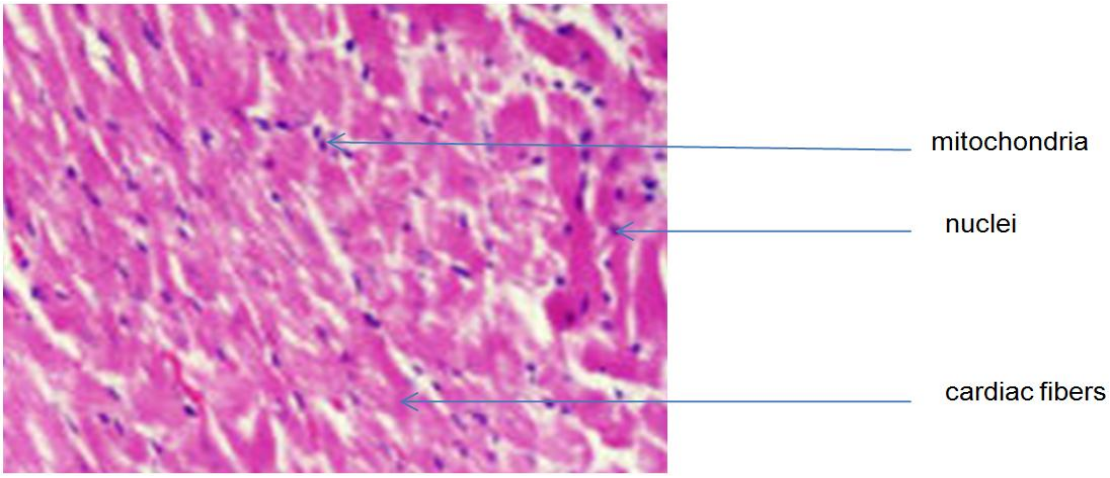

Fig. 1. Photomicrograph (H&E×100) of rat section in normal control (normal fibers)

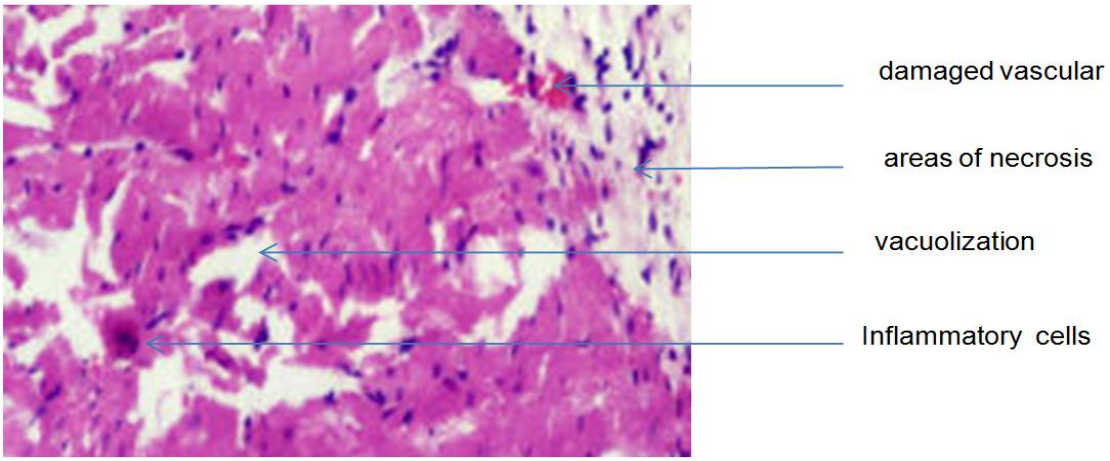

Fig. 2. Photomicrograph (H&E×100) of rat section treated with dox at 2.5 mg/kg showing abundant areas of necrosis and aggregation of acute inflammatory cells and damaged vascular spaces

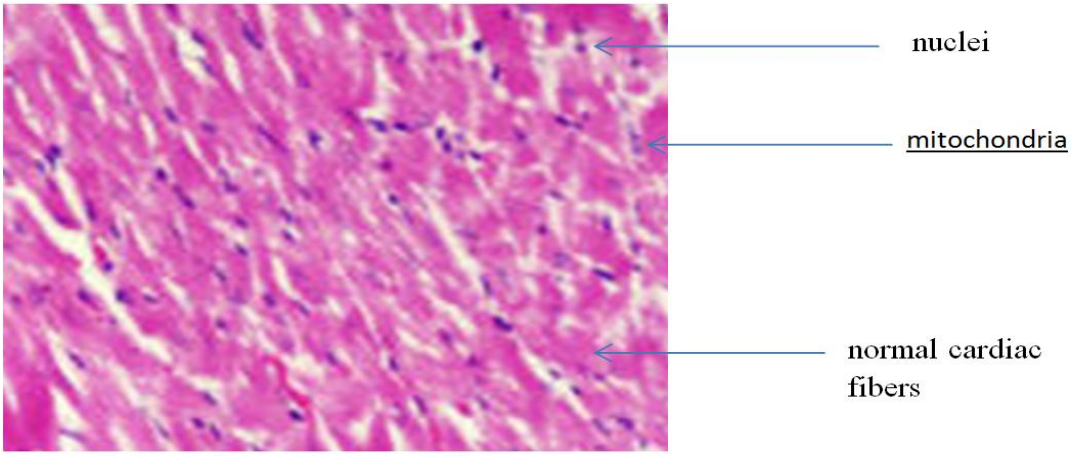

Fig. 3. Photomicrograph (H&E×100) of rat section treated with Tanopati at 10 mg/kg showing normal fibers and nuclei.

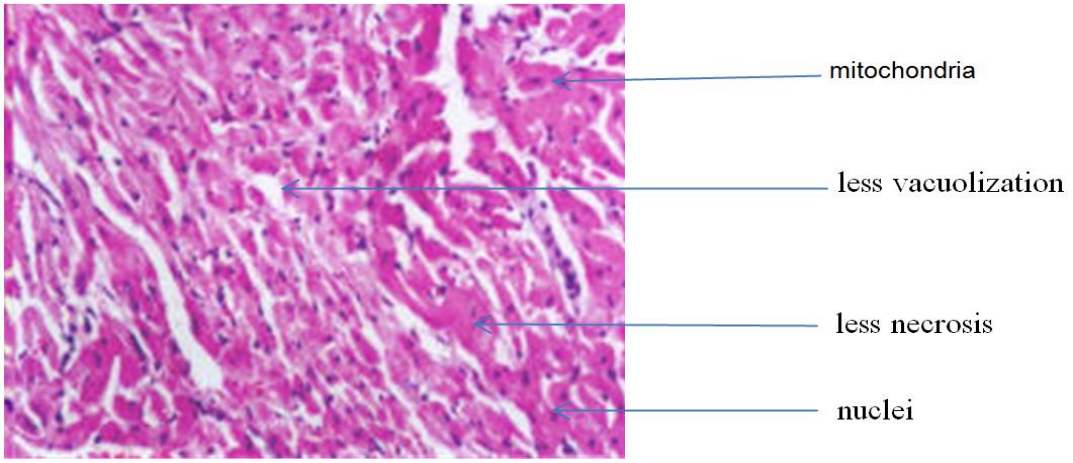

Fig. 4. Photomicrograph (H&E×100) of rat section treated with Tanopati at 10 mg/kg and dox at 2.5 mg/kg showing less necrosis, less vacuolization of the cytoplasm

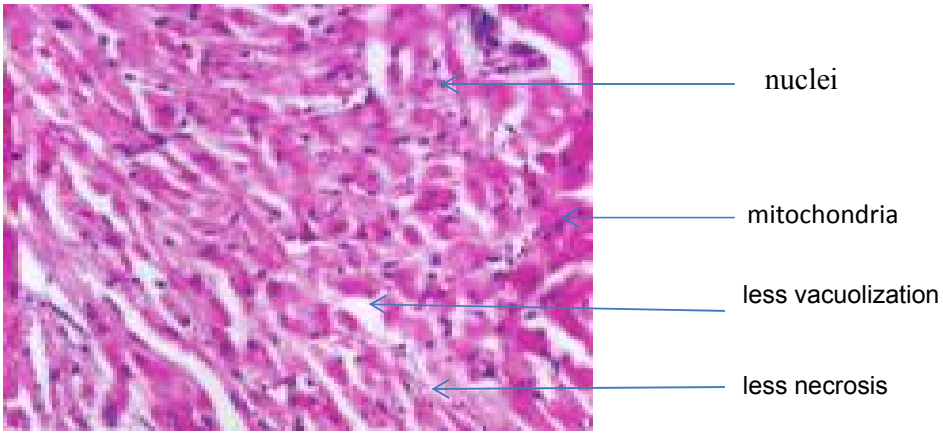

Fig. 5. Photomicrograph (H&E×100)of rat section treated with vit E at 100 mg/kg and dox at 2.5 mg/kg showing less necrosis, less vacuolization of the cytoplasm

4. DISCUSSION

Our results confirmed that a cumulative dose of dox (15 mg/kg) induced cardiotoxicity in rats as evidenced by decreased in heart weight, increased levels of biomarker enzymes and loss of cardiomyocytes. Frequent administration of dox has been shown to cause cardiomyopathic changes in patients as well as in a variety of animal models. Administration of Tanopati, a polyherbal recipe and vitamin E reduced dox-induced mortality in rats.

The experimental study reveals severe biochemical changes as well as oxidative damage in the cardiac tissue after the chronic treatment with dox (cumulative dose of 15 mg/kg body weight) [19]. Doxorubicin is a well-known cardiotoxic agent due to its ability for the destruction of myocardial cells. As a result of this, lactate dehydrogenase (LDH), creatine kinase (CK) and CKMB were released into blood stream and served as the diagnostic markers of myocardial tissue damage. The amount of these cellular enzymes present in the blood reflects the alteration in plasma membrane integrity and/or permeability.

In the present study, dox-treated rats showed significant elevation in the levels of these diagnostic marker enzymes (LDH, CK and CKMB). Moreover, elevated levels of these enzymes are an indicator of the severity of dox-induced myocardial damages, which is in line with an earlier report [20]. The prior administration of Tanopati and vitamin E showed significant reduction in doxo-induced elevated serum marker enzymes. This reduction confirms that Tanopati is responsible for maintenance of normal structural and architectural integrity of cardiac myocytes, by restricting the leakage of these enzymes, which can be accounted for membrane-stabilizing property of Tanopati. Similar results have been observed by Koti et al. [21] and Ayaz et al. [22]. Previously, dox related cardiotoxicities are well documented; dox is metabolically reduced to highly reactive free radicals, which generates superoxide and hydrogen peroxide. These highly toxic free radicals cause lipid peroxidation, inhibition of long chain fatty acids [23,24] and cause damage to cellular components. In rat treated with dox, we found significant increase in heart tissue MDA levels, suggesting increased lipid peroxidation and decreased in levels of GSH, SOD, and CAT. These events are associated with development of a variety of sub-cellular changes in the myocardium, typical of dox-induced cardiac injury. Pretreatment with Tanopati (10 mg/kg) and vitamin E (100 mg/kg) efficiently counteracted the dox-induced cardiac tissue damage by significant decrease in MDA and increase in GSH, SOD, and CAT. But the effect of the vitamin E taken as antioxidant of reference is slightly higher than that of tanopati Histological and bio chemical evidence for the cardioprotective effect of vitamin E in dox induced cardiotoxicity in rats was studied and showed that Vitamin E treatment helped to de-crease the levels of CPK-MP and LDH that were increased due to myocardial damage caused by dox. Increased Vitamin E levels in serum have been reported to decease lipid peroxidation and decease protein kinase C [25-27].

Histopathological report suggests that Tanopati pretreated group attenuates the dox-induced loss of myofibrils, vacuolization of the cytoplasm, and swelling of mitochondria. The histopathological changes observed in the dox-treated rats were similar to those previously report.

Collectively, biochemical and histopathological results provide a possible and potential cardioprotection against doxorubicin cardiotoxicity. Therefore, the antioxidant mechanism of Tanopati may include its well-known ingredients possessing antioxidant activity pointed out in our earlier work [12] and other author's [28-34], which protects the cell from degenerative changes. Thus, in this work, Tanopati effectively prevented tissue damage by decreasing the oxidative stress and restoring the antioxidant status.

5. CONCLUSION

Our work has confirmed the cardiotoxicity induced by doxorubicin is in relationship with oxidative stress. On the other hand, the present study suggests that Tanopati may be considered as a potentially useful candidate to limit free radical-mediated myocardial injury. This activity is a good property for this polyherbal drug used traditionally to cure hypertension, an illness depending of heart integrity and function justify its use in the treatment of hypertension. However, further studies are warranted to characterize the active phytoconstituents involved in the cardioprotection.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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