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Potential interaction of green tea extract with hydrochlorothiazide against doxorubicin-induced myocardial damage

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ABSTRACT

Background: Treatment of ischemic hypertensive patients with hydrochlorothiazide can precipitate cardiac arrhythmias. Green tea, by virtue of its antioxidant potential, is responsible for cardio-protective activity. **Objective:** The present study was under taken to evaluate the pharmacodynamic interaction of green tea extract with hydrochlorothiazide (HCTZ) against doxorubicin (DOX)-induced myocardial toxicity. **Materials and Methods:** Rats were treated with high (500 mg/kg, p.o.) and low (100 mg/kg, p.o.) dose of green tea extract in alone and interactive groups for 28 days. Standard, high and low dose of interactive groups received hydrochlorothiazide (10 mg/kg, p.o.) for the last 7 days. Apart from normal controls, all other groups were subjected to DOX (3 mg/kg, i.p.) toxicity on Days 1, 7, 14, 21 and 28, and the effect of different treatments was evaluated by changes in electrocardiographic parameters, serum biomarkers and tissue antioxidant levels. Apart from that, lipid profile and histological studies were also carried out. **Results:** Compared with the DOX control group, both high and low dose of green tea exhibited a significant decrease in serum biomarkers and increase in tissue antioxidant levels. Green tea treatment was also responsible for significant improvement in ECG parameter, lipid profile and histological score. Incorporation of high and low dose of green tea with HCTZ exhibited significant protection compared with the HCTZ alone treated group. **Conclusion:** The present findings clearly suggest that the green tea extract dose-dependently reduces DOX-induced myocardial toxicity. Green tea when combined with HCTZ can reduce the associated side-effects and exhibits myocardial protection.

Key words: Doxorubicin, green tea, hydrochlorothiazide

INTRODUCTION

Doxorubicin (DOX), an anthracycline derivative, is one of the most effective and useful anti-neoplastic agents commonly used for the treatment of a variety of tumors including solid and malignant lymphoma. However, its clinical use is restricted due to its cardiotoxic effects. It is associated with the development of ischemic conditions in the heart and other cardiac adverse events, such as congestive heart failure and left ventricular dysfunction. The mechanism of DOX-induced cardiotoxicity is not completely understood, but recent studies have postulated the involvement of oxygen free radicals in the development of cardiotoxicity. DOX, by the virtue of its semi-quinone group, has been reported to increase the generation of superoxide radicals and hydrogen peroxide, thereby damaging the myocardial tissue.[1-3]

For the treatment of ischemic hypertensive patients, hydrochlorothiazide (HCTZ), a well-known diuretic, is often avoided as monotherapy. HCTZ is responsible for marked potassium loss. It has been observed that a mild to moderate level of hypokalemia may lead to the development of cardiac arrhythmias.[4] The combination of HCTZ with angiotensin-converting enzyme inhibitor-I, aldosterone antagonist or angiotensin type-I receptor blocker can be beneficial. Hence, the search for concurrently administered safe therapeutic medicament continues, which can ameliorate the HCTZ-induced hypokalemia in patients with ischemic heart diseases.[4,5]

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From the dawn of civilization, it has been evident that herbs and herb-based therapy played an important role to treat different diseased conditions and to improve the quality of life.[6] In recent trend, herb–drug interaction is a thirst area of research as it may influence the pharmacokinetic and pharmacodynamic profiles of each other and can mimic, magnify or oppose the action of each other.[7,8]

Green tea is one of the most consumed beverages in the world. It is obtained from the non-fermented leaves of *Camellia sinensis* belonging to family Theaceae, which contains more catechins than black tea or oolong tea. Because of the presence of high levels of catechin, certain minerals and vitamins increase the antioxidant potential of this type of tea. Since ancient times, green tea has been considered as a healthful beverage by the traditional Chinese medicine. Recent studies have reported that green tea may contribute to the reduction in risk of cardiovascular diseases and some forms of cancer as well as to the promotion of oral health. It has antibacterial, antiviral, neuro-protective and anti-fibrotic properties. It is reported that green tea protects from solar ultraviolet rays and increases bone mineral density.[9,10]

Green tea has been reported to prevent left ventricular hypertrophy, hypertension, cardiovascular damage and endothelial dysfunction.[11,12]

Green tea has an anti-atherosclerotic property and can attenuate diabetes-induced cardiovascular complications by ameliorating myocardial collagen characteristics.[13,14]

The antioxidant property of green tea protects the cardiomyocyte cytoskeleton from oxidative injury.[15,16] Apart from that, green tea can enhance cardiac contractility by modulating myofilament Ca^{2+} sensitivity.[17] It has been reported that green tea is able to prevent DOX-induced cardiac abnormalities and pathological changes.[18] In the modern Ayurvedic text, it has been documented that green tea can improve lipoprotein levels by reducing low-density lipoprotein (LDL) and total cholesterol. Moreover, green tea can improve the ratio of LDL cholesterol to high-density lipoprotein cholesterol. Green tea reduces blood pressure and body weight.[19]

Till now, no study has been carried out to investigate the combined effect of green tea and HCTZ in DOX-induced myocardial ischemic conditions. The present study has been designed to evaluate the effect of green tea and HCTZ against DOX-induced myocardial toxicity.

MATERIALS AND METHODS

Chemicals

All chemicals used were of analytical grade and purchased from standard companies. Pure sample of HCTZ was gifted by the Bangalore Test House (Bangalore, India). Biochemical kits were procured from Crest Biosystems (Goa, India).

Experimental animals

Healthy adult Wistar albino rats of either sex weighing 175–250 g were housed in polypropylene cages, maintained under standardized conditions (12-h light: Dark cycles, 25° ±5°C) with paddy husk bedding at the Central Animal House, Shree Devi College of Pharmacy, Mangalore, India, were provided with standard pellet food and had free access to purified drinking water. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India were followed and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study (SDCP/IAEC-19/2012-13).

Plant materials

Green tea (*Camellia sinensis*) leaves were purchased in the month of June, 2013 from the local market of Mangalore bearing the brand name GREEN TEA, manufactured by New Hilltop Traders (Vandiperiyar, Kerala, India). The authentication was performed by Dr. Neoline J. Pinto, H.O.D., Department of Botany, St. Agnes College, Mangalore (SAC/MNG/SMP/Drug/2013-06/52). The aqueous extract was prepared by mixing Green tea leaves gently in distilled water maintained at 70–80°C for 30 min. Thereafter, it was filtered and evaporated in the same temperature to get a thick gummy mass. The yield was found to be 24.76% (W/W). The extract was freshly dissolved in distilled water before giving each dose to the animals.

Phytochemical estimations of the extract

Aqueous extract of Green Tea (GTE) was subjected to qualitative analysis to investigate the presence of various phytochemical constituents like alkaloids, glycosides, steroids, flavonoids, gallic tannins, catecholic tannin, terpenoid and saponins.[20,21]

Acute toxicity study

The acute toxicity study was carried out according to the OPPTS (Office of Prevention, Pesticide and Toxic Substance) guidelines following the limit test procedure.[22]

Mice were fasted overnight prior to the studies and then divided into two groups of three each. Test doses of 2 g/kg body weight and 5 g/kg body weight were given orally to either group of mice and then observed for 72 h for mortality. $1/10^{th}$ and $1/50^{th}$ of the maximum safe dose corresponding to 500 and 100 mg/kg orally were selected as high and low doses, respectively.

Experimental protocol

The animals were divided into seven different treatment groups of eight animals each. Group I and Group II received saline for 4 weeks and were termed as normal control and DOX control, respectively; Group III received HCTZ (10 mg/kg) for the last 7 days;[4] and Groups IV and V received GTE 100 mg/kg (GTE-100) and 500 mg/kg (GTE-500) for 28 days, respectively. Group VI and Group VII were treated with GTE-100 and GTE-500, respectively, along with HCTZ. All treatments were performed by the oral route. Except Group I, all other groups were subjected to DOX toxicity with a dose of 3 mg/kg i.p. on Days 1, 7, 14, 21 and 28, which is a cumulative dose of 15 mg/kg.[18]

General observations

During the treatment period, water consumption, food consumption and mortality rates were noted.[23]

Electrocardiographic studies

Forty-eight hours after the last treatment, the animals were anesthetized with the combination of ketamine (75 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.). Leads were attached to the dermal layer of both the front paws and the hind legs and recordings were made with the help of a digital physiograph (model no- DI-2, INCO, Ambala city, India). The changes in heart rate, QRS interval, QT interval and RR interval were determined.[22]

Oxidative marker enzyme assay

Forty-eight hours after the last treatment, blood was collected for the separation of serum and analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), creatine kinase-MB (CK-MB), creatine kinase-NAC (CKNAC) and lactate dehydrogenase (LDH). Estimation of marker enzymes was performed by using commercial kits with the help of a semi-autoanalyzer (model: Prietest touch, Robonik India Pvt. Ltd. Navi Mumbai, Maharashtra, India).

Then, the animals were sacrificed by mild ether anesthesia. Four hearts from each group were homogenized with sucrose solution (0.25 M) for estimations of superoxide dismutase (SOD), catalase and thiobarbituric acid reactive species (TBARS).[22,23]

Lipid profile assay

Serum cholesterol and triglyceride levels were measured by commercial kits with the help of a semi-autoanalyzer.[24]

Histological analysis

Heart sections were prepared from the remaining four hearts in each group, stained with hematoxylin and eosin (H and E) and the changes in histology were observed. The myocardial damage was determined by the scoring method depending on the severity as follows: No change = 0 score; mild = 1 score (focal myocytes damage or small multifocal degeneration with slight degree of inflammation); moderate = 2 score (extensive myofibrillar degeneration) and marked = 3 score (necrosis with diffuse inflammation).[22]

Statistical analysis

Results are expressed as mean ± SE. Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by the Tukey–Karmer multiple comparison tests. $P < 0.05$ was considered significant.

RESULTS

Preliminary phytochemical investigation

The preliminary phytochemical investigation of the GTE extract showed the presence of alkaloids, flavonoids, steroids, gallic tannins and catecholic tannins. The percentage yield of GTE was found to be 24.76%.

General observation and mortality

The general appearance of all groups of animals was recorded throughout the study. In the later days, DOX-treated animals developed a pink tinge and the animals' fur became scruffy. These rats also had red exudates around the eyes and nose, soft watery feces and enlargement of the abdomen. These conditions were more severe at the end of the study period.

The DOX control and HCTZ-treated groups showed a significant decrease in their feed and water consumption during the drug treatment period as compared with the normal control group. It was improved significantly in the high and low dose of GTE and their combination groups with HCTZ compared with the DOX control. Low and high dose of GTE combined with HCTZ demonstrated significant improvement in food and water consumption compared with the HCTZ alone treated group [Table 1].

Table 1: Effect on water consumption, food consumption and mortality in DOX-induced myocardial toxicity

Treatment	Water consumption (mL/rat/day)	Food consumption (g/rat/day)	Mortality (death/ total)
Normal control	22.45±0.45	23.61±0.81	0/8
DOX control	11.40±0.36***	12.56±0.56***	4/8
HCTZ	12.46±0.71***	14.67±0.62***	3/8
GTE-100	17.34±62###	22.45±0.23###	0/8
GTE-500	18.72±0.81###	23.81±0.85###	0/8
GTE-100+HCTZ	15.72±0.92**#♦	18.91±0.71*#♦♦	1/8
GTE-500+HCTZ	16.67±0.37*#♦	19.94±0.59*##♦♦	1/8

All values are mean±SEM, n=8, ***P<0.001, **P<0.01, *P<0.05 when compared with the normal control, ###P<0.001, ##P<0.01, #P<0.05 compared with the DOX control and ♦♦P<0.01, ♦P<0.05 compared with HCTZ, GTE-100 (Green tea extract-100 mg/kg), GTE-500 (Green tea extract-500 mg/kg) and HCTZ (hydrochlorothiazide - 10 mg/kg), DOX=Doxorubicin

Effect on electrocardiographic parameters

The DOX control group demonstrated a significant increase in QT segment, PR interval and QRS interval and significant decrease in heart rate and RR interval compared with the normal control.

GTE, in a dose-dependent manner, rectified DOX-induced change in ECG to normal. GTE, especially with the dose of 500 mg/kg when combined with HCTZ, demonstrated significant improvement in ECG parameter compared with the HCTZ alone treated group [Table 2].

Serum enzyme biomarkers

The DOX-treated group demonstrated significant increase in serum AST, ALT, ALP, CK-MB, CK-NAC and LDH values compared with the normal control. Treatment groups such as GTE-100, GTE-500, GTE-100 + HCTZ and GTE-500 + HCTZ showed a significant decrease in AST, ALT, ALP, CK-MB, CK-NAC and LDH values compared with toxic control. The GTE-100 + HCTZ and GTE-500 + HCTZ treated groups showed a significant decrease in AST, ALT, ALP, CK-MB, CK-NAC and LDH values compared with the HCTZ alone treated group [Table 3].

Effect on SOD and catalase

The SOD and Catalase activities were reduced significantly in DOX control compared with normal control. Experimental groups such as GTE-100, GTE-500 and their combination with HCTZ resulted in significant improvement in SOD and Catalase activity compared with the DOX-treated group. Moreover, GTE-100 and GTE-500 incorporated with HCTZ represented a significant increase in SOD and Catalase values compared with the HCTZ alone treated group [Table 4].

Effect on TBARS

DOX treatment was responsible for a significant increase in the TBARS levels compared with the normal control. GTE treatment in a dose-dependent manner demonstrated significant reduction in TBARS levels compared with the DOX control group. Incorporation of HCTZ during the last 7 days of GTE treatment resulted in a significant fall in the TBARS levels compared with HCTZ alone [Table 4].

Effect on Lipid Profile

Significant incremental values were found for triglycerides and cholesterol levels in case of the DOX-intoxicated group compared with normal control. Treatment with GTE-100, GTE-500 and their combination with HCTZ demonstrated significant reduction in triglycerides and cholesterol levels compared with the DOX control group. Concurrent administration of GTE-500 with HCTZ showed a significant decrease in the lipid profile compared with HCTZ alone [Table 4].

Table 2: Effect on hemodynamic findings and electrocardiograph patterns against DOX-induced myocardial toxicity

Treatments	Heart rate (beats/min)	QT interval (ms)	RR interval (ms)	PR interval (ms)	QRS interval (ms)
Normal control	351.91±0.47	59.83±0.39	19.47±0.72	51.85±0.11	17.42±0.39
DOX control	267.29±0.92***	97.91±0.64***	15.11±0.29*	76.78±0.95**	20.83±0.62*
HCTZ	290.30±0.38**$^{\#}$	76.73±0.61**$^{\#\#}$	16.23±0.96*	69.23±0.48*	19.45±0.89*
GTE-100	328.75±0.41*$^{\#\#}$	69.39±0.91*$^{\#\#}$	17.91±0.42*$^{\#}$	61.42±0.39*$^{\#}$	18.92±0.29*$^{\#}$
GTE-500	345.25±0.83$^{\#\#}$	61.20±0.71*$^{\#\#\#}$	18.89±0.19$^{\#\#}$	57.98±0.81$^{\#\#}$	17.56±0.33$^{\#\#}$
GTE-100+HCTZ	301.49±0.76**$^{\#}$	74.18±0.49**$^{\#\#}$	16.79±0.32*	65.91±0.79*	19.29±0.79*
GTE-500+HCTZ	316.69±0.45**$^{\#\#\bullet}$	70.25±0.31**$^{\#\#\bullet}$	17.98±0.79*$^{\#\bullet}$	62.77±0.35*$^{\#\#\bullet}$	18.39±0.91*$^{\#\bullet}$

All values are mean±SEM, n=8, ***P<0.001, **P<0.01, *P<0.05 compared with the normal control, $^{\#\#\#}P$<0.001, $^{\#\#}P$<0.01, $^{\#}P$<0.05 compared with the DOX control and $^{\bullet}P$<0.05 compared with HCTZ, GTE-100 (Green tea extract-100 mg/kg), GTE-500 (Green tea extract-500 mg/kg) and HCTZ (hydrochlorothiazide - 10 mg/kg), DOX=Doxorubicin

Table 3: Effect on serum marker enzymes against DOX-induced myocardial toxicity

Treatment	Blood serum level, U/L					
	CK-MB	CK-NAC	LDH	AST	ALT	ALP
Normal control	79±0.82	85.56±0.17	156.54±0.71	78.95±0.36	35.96±0.83	95.82±0.72
DOX control	347±0.79***	391.39±0.59***	580.73±0.89***	234.56±0.56***	169.27±0.32***	390.62±0.96***
HCTZ	294±0.53***	272.59±0.62***	486.29±0.52	191.82±0.81***	138.93±0.79***	280.74±0.78***
GTE-100	178±0.38*$^{\#\#\#}$	189.92±0.79*$^{\#\#\#}$	237.11±0.45*$^{\#\#\#}$	113.99±0.72$^{\#\#\#}$	89.72±0.52$^{\#\#\#}$	160.45±0.11*$^{\#\#\#}$
GTE-500	123±0.10$^{\#\#\#}$	158.19±0.38$^{\#\#\#}$	192.91±0.69$^{\#\#\#}$	89.43±0.97$^{\#\#\#}$	55.76±0.39$^{\#\#\#}$	120.39±0.24$^{\#\#\#}$
GTE-100+HCTZ	259±0.29**$^{\#\bullet}$	223.39±0.41**$^{\#\bullet}$	328.29±0.36**$^{\#\bullet}$	157.53±0.38**$^{\#\bullet}$	105.54±0.64**$^{\#\bullet}$	215.92±0.61**$^{\#\bullet\bullet}$
GTE-500+HCTZ	206±0.31*$^{\#\#\bullet\bullet}$	198.47±0.20*$^{\#\#\bullet\bullet}$	276.19±0.42*$^{\#\#\bullet\bullet}$	139.49±0.73*$^{\#\#\bullet\bullet}$	94.39±0.29*$^{\#\#\bullet\bullet}$	190.67±0.70*$^{\#\#\bullet\bullet}$

All values are mean±SEM, n=8, ***P<0.001, **P<0.01, *P<0.05 compared with normal control, $^{\#\#\#}P$<0.001, $^{\#\#}P$<0.01, $^{\#}P$<0.05 compared with DOX control and $^{\bullet\bullet}P$<0.01, $^{\bullet}P$<0.05 compared with HCTZ, GTE-100 (Green tea extract-100 mg/kg), GTE-500 (Green tea extract-500 mg/kg) and HCTZ (hydrochlorothiazide - 10 mg/kg), DOX=Doxorubicin, CK-MB=Creatine kinase-MB, CK-NAC=Creatine kinase-NAC, LDH=Lactate dehydrogenase, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline phosphate

Effect on histological score

Myocardial integrity was disturbed significantly by doxorubin, which was evident by significant increase in histological score compared with normal control. DOX administration was responsible for extensive myofibrillar degeneration, marked diffuse inflammation and increased interstitial space. The treatment groups treated with GTE alone and incorporated with HCTZ showed significant reduction in histological score compared with the DOX control group. Combination therapy of HCTZ with high dose of GTE (500 mg/kg) demonstrated significant reduction in histological score compared with HCTZ alone. The protective effect of combined therapy of HCTZ with high dose of GTE was demonstrated with least multifocal degeneration and mild inflammation with reduction in interstitial space [Table 4] [Figure 1].

DISCUSSION

The aim of the present study was to elucidate the pharmacodynamic interaction of GTE with HCTZ against DOX-induced myocardial injury.

Observed results suggested that GTE (100 and 500 mg/kg, p.o.) showed beneficial results dose dependently. Apart from that, GTE when combined with HCTZ indicated better results compared with the HCTZ alone treated group against DOX-induced myocardial injury.

HCTZ alters the renal tubular mechanisms of electrolyte reabsorption. The direct action of HCTZ is responsible

Table 4: Effect on serum lipid profile, antioxidants in HTH and histological score against DOX-induced myocardial toxicity

Treatment	Blood serum level, mg/dL		Heart tissue homogenate (units/mg of protein)		
	Cholesterol	Triglycerides	SOD	Catalase	TBARS
Normal control	71.39±0.19	159.83±0.45	30.34±0.34	21.45±0.56	27.38±0.39
DOX control	125.82±0.52***	361.81±0.19***	12.82±0.78***	8.82±0.39***	69.35±0.91***
HCTZ	101.29±0.67***	310.65±0.28***	14.56±0.45***#	12.98±0.20***#	55.73±0.25***#
GTE-100	89.38±0.83***##	239.39±0.18***##	24.39±0.56**###	17.65±0.49**###	38.29±0.46**###
GTE-500	76.49±0.31###	187.53±0.72###	29.20±0.97###	22.20±0.37###	32.32±0.69###
GTE-100+HCTZ	96.22±0.96**##	285.60±0.34**#	19.72±0.29***###••	15.39±0.19***###••	41.56±0.20***###••
GTE-500+HCTZ	75.31±0.38###••	195.36±0.16###••	21.11±0.49***###•••	16.52±0.25**###•••	47.25±0.19***###•••

All values are mean±SEM, *n*=8, ***P*<0.001, **P*<0.01, **P*<0.05 when compared with normal control; ###*P*<0.001, ##*P*<0.01, #*P*<0.05 compared with DOX control and •••*P*<0.001, ••*P*<0.01, •*P*<0.05 compared with HCTZ, GTE-100 (Green tea extract-100 mg/kg), GTE-500 (Green tea extract-500 mg/kg) and HCTZ (hydrochlorothiazide - 10 mg/kg), DOX=Doxorubicin, SOD=Superoxide dismutase, TBARS=Thiobarbituric acid reactive species, HTH=Heart tissue homogenate

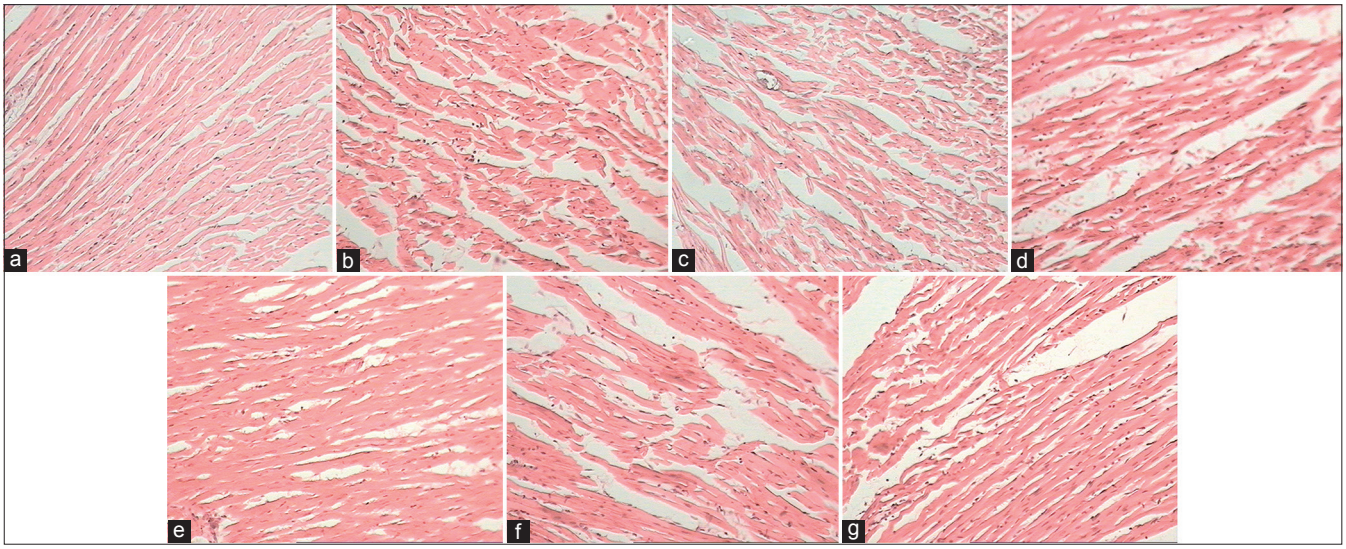

Figure 1: (a) Hematoxylin and eosin (H and E) (x400)-stained microscopic section of normal control group (normal texture of cell). (b) H and E (x400)-stained microscopic section of the doxorubicin (200 mg/kg, i.p.) control group (necrotic cells with degeneration of myofibril, increased interstitial space, diffuse inflammation). (c) H and E (x400)-stained microscopic section of the hydrochlorothiazide (10 mg/kg, p.o.) group (extensive myofibrillar degeneration, increased interstitial space, slight degree of inflammation). (d) H and E (x400)-stained microscopic section of Green tea extract (GTE)-100 (100 mg/kg, p.o.) and doxorubicin (200 mg/kg, i.p.) (less interstitial space, myofibrillar degeneration). (e) H and E (x400)-stained microscopic section of GTE-500 (500 mg/kg, p.o.) and doxorubicin (200 mg/kg, i.p.) (small multifocal degeneration, slight inflammation). (f) H and E (x400)-stained microscopic section of GTE-100 (100 mg/kg, p.o.) and hydrochlorothiazide (10 mg/kg, p.o.) and doxorubicin (200 mg/kg, i.p.) (extensive myofibrillar degeneration, inflammation). (g) H and E (x400)-stained microscopic section of GTE-500 (500 mg/kg, p.o.) and hydrochlorothiazide (10 mg/kg, p.o.) and doxorubicin (200 mg/kg, i.p.) (small multifocal degeneration, slight inflammation, fall in interstitial space)

for an increase in the excretion of sodium and chloride. The indirect action causes reduction in plasma volume, consequent increase in urinary potassium loss, plasma renin activity and aldosterone secretion and decrease in serum potassium loss. It has been observed that in patients with cardiac ischemia, heart failure or left ventricular hypertrophy, the likelihood of cardiac arrhythmia is increased because of mild-to-moderate hypokalemia. It has been reported that HCTZ-induced hypokalemia is responsible for the increase in serum biomarker levels, such as lactate dehydrogenase (LDH) and creatinine kinase-MB (CKMB) and decrease in heart tissue antioxidant levels like super oxide dismutase (SOD) and catalase in rats. Therefore, it is proven that their undesirable metabolic consequences have been suspected to contribute an increase in cardiovascular morbidity and mortality. Hence, search for concurrently administered safe therapeutic medicament continues, which can ameliorate the hypokalemia in patients with ischemic heart diseases.[4,5]

Camellia sinensis (Green tea) has a rich source of polyphenols that is responsible for strong free radical-scavenging activity. Green tea has reported its potency against cardiovascular disease risk factors. It reduces body weight by interfering within the sympatho-adrenal system, reduces fatty acid synthesis and decreases cholesterol absorption. It possesses antithrombotic activity by inhibiting platelet aggregation. Green tea inhibits low-density lipid oxidation, reduces adhesion molecule expression and reduces systolic as well as diastolic blood pressures.[25]

In a DOX-induced myocardial injury model, toxicity was induced by treating the experimental animals with a total cumulative dose of 15 mg/kg by the intraperitoneal route. DOX is responsible for the development of myocardial toxicity by generation of free radicals such as superoxide and hydroxyl radicals. These generated free radicals are responsible for the damage of various intracellular components. Apart from that, presence of low levels of free-radical detoxifying enzymes/molecules like SOD and catalase makes the myocytes more susceptible to free radical-induced injury. DOX has a higher affinity for the phospholipid component of the mitochondrial membrane in myocardial cells, which leads to DOX accumulation in heart tissue.[26,27] In this present study, it has been demonstrated that significant decrease in SOD and catalase activity and increase in TBARS in DOX- and HCTZ-treated groups indicates severe myocardial toxicity. GTE dose dependently (100 mg/kg and 500 mg/kg) restored SOD, catalase and TBARS levels. Observed results suggested that GTE when combined with HCTZ showed better protection compared with HCTZ alone by increase in SOD and catalse and decrease in TBARS levels.

DOX-induced cardiotoxicity is very critical as it is responsible for alterations in the energy metabolism leading to abnormal contractile functioning, congestive heart failure and left ventricular dysfunction.[3,28]

DOX-induced cardiotoxicity is responsible for leakage of marker enzymes from mitochondria, resulting in elevated serum levels.[24] It has been observed in the present study that DOX treatment causes elevated levels of serum enzymes and markers such as ALT, AST, ALP, LDH, CK-MB and CK-NAC, which indicate the loss of myocardial cell integrity. GTE in both high (500 mg/kg) and low doses (100 mg/kg) reflected reduction in marker enzyme levels that encourage myocardial protection. GTE when incorporated with HCTZ showed better protection compared with the HCTZ alone treated group.

It has been reported that DOX causes an increase in ST and QT interval and a decrease in heart rate of ECG pattern, which are some of the common witnesses of myocardial injury.[18]

In this study, DOX treatment caused prolongation of PR, QRS interval and QT segment. These abnormal electrocardiographic changes and decrease in heart rate indicated myocardial toxicity. GTE in both the doses restored the electrocardiographic parameters. GTE, especially 500 mg/kg, when incorporated with HCTZ substantially reflected better protection in terms of electrocardiographic parameters compared with the HCTZ alone treated group.

DOX interferes with the metabolism and biosynthesis of lipids, thereby increasing the cholesterol and triglyceride levels.[24]

In our present study also, the DOX control group showed significant increase in total cholesterol and triglyceride levels. Green tea treatment improved the lipid profile by decreasing cholesterol and triglyceride levels. The possible mechanisms by which GTE can exert a cholesterol-lowering effect are reducing the absorption of dietary and biliary cholesterol and promoting its fecal excretion.[29]

GTE, particularly at the 500 mg/kg dose, when incorporated with HCTZ demonstrated significant improvement in lipid profile compared with HCTZ alone.

A histological study in DOX-induced cardiotoxicity supported the findings of other parameters analyzed in different treatment groups. For the normal heart, myocardial fibers were found to be of uniform size, shape and configurations with no inflammatory cell infiltrates. DOX treatment was evident with enormous changes in the

myocardial cell associated with degeneration of myocardial tissue, vacuolization of the cardiomyocytes, infiltration of inflammatory cells and myofibrill loss. Treatment with GTE dose dependently inhibited DOX-induced cardiac damage by decreasing fragmentation of the myofibrils and inflammation. GTE, predominantly in the higher dose (500 mg/kg), was able to retrieve the pathological changes associated with HCTZ in myocardial cells.

CONCLUSION

From this present study, it can be concluded that GTE exhibited a dose-dependent protection against DOX-induced cardiotoxicity. Moreover, combination of GTE with HCTZ demonstrated significant reduction in myocardial side-effects associated with HCTZ. This observation can be very important for cancer patients with hypertension and myocardial ischemic conditions, where HCTZ cannot be given as monotherapy due to potential myocardial side-effects. Future studies can be carried out to establish the fact clinically.

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