ORIGINAL ARTICLE

Effect of chronic administration of green tea extract on chemically induced electrocardiographic and biochemical changes in rat heart

Leena J. Patil, Sunil B. Bothara, Balaraman R.
Pharmacy Department, Faculty of Technology and Engineering, M.S. University of Baroda, Baroda - 390 001, Gujarat, India

Many chemicals induce cell-specific cytotoxicity. Chemicals like doxorubicin induce oxidative stress leading to cardiotoxicity causing abnormalities in ECG and increase in the biomarkers indicating toxicity. Green tea extract (GTE), *Camellia sinensis* (Theaceae), is reported to exert antioxidant activity mainly by means of its polyphenolic constituent, catechins. Our study was aimed to find out the effect of GTE (25, 50, 100 mg/kg/day p.o. for 30 days) on doxorubicin-induced (3 mg/kg/week, i.p. for 5 weeks) electrocardiographic and biochemical changes in rat heart. It is observed that GTE administered rats were less susceptible to doxorubicin-induced electrocardiographic changes and changes in biochemical markers like lactate dehydrogenase (LDH), creatine kinase (CK), and glutamic oxaloacetate transaminase (GOT) in serum, and superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH), membrane bound enzymes like Na^+K^+ATPase, Ca^{2+}ATPase, Mg^{2+}ATPase and decreased lipid peroxidation (LP) in heart tissue, indicating the protection afforded by GTE administration.

Key words: Antioxidant, catechins, doxorubicin, electrocardiogram, green tea

INTRODUCTION

It has been widely reported that doxorubicin, an anthracycline antibiotic for cancer treatment, causes cardiotoxicity due to the production of free radicals.[1] The clinical effectiveness of doxorubicin treatment for several cancers is affected by the dose-limiting side effect, cardiotoxicity.[2] In the past, several studies concluded that antioxidants such as α-tocopherol (αTC)[3] and a-phenyl-*tert*-butyl-nitrone[4] afforded protection from doxorubicin-induced myocardial injury without affecting its antineoplastic activity.

Polyphenols are plant metabolites occurring widely in plant foods that possess outstanding antioxidant and free radical scavenging properties.[5] Green tea is an excellent source of polyphenol antioxidants, particularly of the group known as green tea catechins (GTCs).[6] Green tea reduces iron-induced lipid peroxidation (LP) in brain homogenates as well as in cultured C6 astrocytes and lung cells.[7] In addition, green tea has also been shown to reduce the formation of the spin-adducts of hydroxyl radicals and hydroxyl radical to induced DNA strand breakage *in vitro*.[8] Green tea has been found to have inhibitory effects on chemical-induced lung tumourigenesis.[9] There is also considerable epidemiological evidence suggesting that the consumption of green tea lowers the risk of heart disease as well as several types of cancer incidences, as a result of these antioxidant mechanisms.[10]

However, to the best of our knowledge, effects of green tea extract (GTE) on doxorubicin-induced electrocardiographic and biochemical changes in rat heart have not been explored yet. Therefore, we investigated the effects of GTE on doxorubicin-induced electrocardiographic and biochemical changes in rat heart to verify the hypothesis that GTE may afford protection due to its catechin contents.

MATERIALS AND METHODS

Chemicals

Standardised powdered, ethyl acetate extract of green tea leaves, C*amellia sinensis* (L.) Kuntze (Theaceae) was a gift sample from Cherain Chemicals, Baroda, India, with a total polyphenolic content of 35%. Doxorubicin injection was a gift sample from Serum Institute of India Ltd., Pune. Epinephrine hydrochloride, superoxide dismutase (SOD), malondialdehyde and catalase (CAT) were purchased from Sigma Aldrich, USA. Reduced glutathione (GSH), 5,5'-dithiobis(-2 nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA) were obtained from HiMedia, India. All other chemicals were of analytical grade.

Animals

Adult albino rats of either sex (Wistar strain) weighing

Address for correspondence: Dr. Leena J. Patil, Bharti Vidyapeeth's College of Pharmacy, CBD-Belapur, Navi Mumbai, Maharashtra, India.
E-mail: leenapatil27@yahoo.com
Received: 20-07-2009; **Accepted:** 26-06-2010; DOI: 10.4103/0973-8258.69175

between 175 and 225 g were used for the study. The animals were fed *ad libitum* with standard pellet diet and had free access to water. All experiments and protocols described in present report were approved by the Institutional Animal Ethics Committee (IAEC) of M. S. University, Baroda, India.

Experimental Protocol

Chemical analysis of green tea extract

Thin layer chromatography (TLC) fingerprint profile of the extract was established using High performance thin layer chromatography (HPTLC). For development of TLC fingerprint, 500 mg of powdered GTE was extracted with 3 × 25 ml of methanol. Extracts were pooled, filtered and concentrated to 25 ml. Suitably diluted stock solution of methanol extract with gallic acid standard solution and catechin were spotted on a pre-coated silica gel G60 F254 TLC plate (E. Merck) using CAMAG Linomat IV Automatic Sample Spotter and the plate was developed in the solvent system of Toluene:ethyl acetate:formic acid (6:6:1). The plate was dried at room temperature and scanned using CAMAG TLC Scanner 3 at UV 254 nm and R_f values and peak area of the resolved bands were recorded. Relative percentage area of each band was calculated from peak areas. The TLC plate was derivatised by spraying with 5% methanol ferric chloride solution for the detection of phenolic compounds.

Groups and treatment schedule

Powdered GTE was reconstituted in distilled water. Doxorubicin injection was dissolved in sterile water for injection. The animals were divided into five groups with six rats in each and they received the following treatment.

Group I: Received distilled water (3 ml/kg/day p.o. for 30 days) and sterile water for injection (1 ml/kg, i.p.) on day 1, 7, 14, 21, 28.

Group II: Doxorubicin injection (3 mg/kg i.p.) on day 1, 7, 14, 21, 28.

Group III: GTE (25 mg/kg/day p.o. for 30 days) and doxorubicin injection (3 mg/kg i.p.) on day 1, 7, 14, 21, 28.

Group IV: GTE (50 mg/kg/day p.o. for 30 days) and doxorubicin injection (3 mg/kg i.p.) on day 1, 7, 14, 21, 28.

Group V: GTE (100 mg/kg/day p.o. for 30 days) and doxorubicin injection (3 mg/kg i.p.) on day 1, 7, 14, 21, 28.

After 48 hours of the last injection of either doxorubicin or vehicle, electrocardiographic changes and changes in serum markers were studied after removal of blood and the heart was excised under euthanasia in chilled Tris buffer (10 mM pH 7.4) for measurement of tissue markers of oxidative stress.

Electrocardiography

ECG was recorded under mild ether anaesthesia through needle electrodes (Lead II) using Biopac MP30 data acquisition system (Biopac Systems, Santa Barbara, CA, USA). The changes in heart rate, QT interval and ST interval were determined from ECG.

Biochemical Parameters

Serum markers

Serum levels of lactate dehydrogenase (LDH) and serum creatine kinase (CK) were determined by using standard kits of Reckon Diagnostic Ltd., India, while glutamic oxaloacetate transaminase (SGOT) was assayed by using standard kit of Span Diagnostic Pvt. Ltd., India.

Biomarkers of the oxidative stress

The excised heart was then weighed and homogenised in chilled Tris buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates were centrifuged at 10,000 × *g* at 4°C for 20 minutes using Remi C-24 high-speed cooling centrifuge. The clear supernatant was used for the assays of malondialdehyde content as indicator of LP,[11] endogenous antioxidant enzymes, SOD,[12] CAT[13] and GSH.[14]

Membrane bound enzymes

The sediment after centrifugation of tissue homogenate was resuspended in ice-cold Tris buffer (10 mM, pH 7.4) to get a final concentration of 10% and was used for the estimation of different membrane bound enzymes such as Na^+K^+ATPase,[15] Ca^{2+}ATPase[16] and Mg^{2+}ATPase[17] and total proteins.[18]

Statistical Analysis

Results of all the above estimations were indicated in terms of mean±SEM. Difference between the groups was statistically determined by analysis of variance (ANOVA) followed by Tukey Kramer multiple comparisons test with the level of significance set at $P \leq 0.05$.

RESULTS

Chemical Analysis

The fingerprint chromatograms are shown in Figure 1. Details of the fingerprint analysis are given in Table 1.

Electrocardiographic Changes

The ECG changes in all the groups are summarised in Table 2. The doxorubicin administration significantly increases ST and QT interval while the heart rate was

Table 1: Details of fingerprint chromatograms of GTE after scanning at 254 nm

Extract	Solvent system	No. of spots
Methanol extract	Toluene:ethyl acetate:formic acid (6:6:1)	8

Rf values: 0.03, 0.12, 0.22, 0.35, 0.43, 0.50, 0.63, 0.68; Relative %: 3.30, 1.84, 33.03, 15.11, 35.09, 4.99, 1.27, 1.05

significantly decreased as compared to control rats. The administration of GTE significantly restores ECG changes towards normal in a dose-dependant manner.

Biochemical Parameters

Serum markers

The levels of serum marker enzymes in all the groups are given in Table 3. Doxorubicin administration significantly increases serum levels of CK, LDH and GOT as compared to control rats. Administration of GTE significantly restores the marker levels towards normal in a dose-dependant manner.

Biomarkers of the oxidative stress

The levels of biomarkers of oxidative stress enzymes in all the groups are presented in Table 4. Doxorubicin administration significantly increases LP while there was a significant decrease in GSH, SOD and CAT levels as compared to control rats. Administration of GTE significantly improves GSH, SOD and CAT levels after doxorubicin administration while LP level changes towards normal values in a dose-dependant manner.

Membrane bound enzymes

Doxorubicin damages cell membrane as evident from significant decrease in the levels of membrane bound enzymes like Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase as compared to control. GTE fails to prevent damage at lower doses and significant improvement was observed at 100 mg/kg dose.

DISCUSSION

The results obtained above indicate that doxorubicin induces pathological changes in ECG and biochemical markers, suggestive of cardiotoxicity and increase in free radical production. These results are consistent with those of earlier studies.[19,20] Further results also lead us to believe that administration of GTE improved the ECG and biochemical marker levels indicating decrease in oxidative stress as evident by increased levels of GSH, SOD and CAT with decreased production of LP. The restoration of membrane bound enzymes like Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase in GTE treated rats is indicative of membrane stabilising protective effect of GTE. These protective effects are also supported by the restoration of serum marker enzymes towards normal levels.

It has been reported that catechins are important constituents of green tea, which are responsible for its antioxidant and protective effects. Further, it is also reported that catechin content depends on several factors that include species, climate, technology used for extraction and conservation.[21] We verified the catechin content of extract using ethyl acetate as medium and found that it contains 35% catechins. In one of the studies involving doxorubicin-induced fatty acid composition modification in cardiomyocytes, it was revealed that only one of the GTE which was rich in catechin contents was able to counteract the detrimental changes and elevation of conjugated dienes.[21] It seems that antioxidant agents can protect the heart from doxorubicin-induced assault as reviewed and confirmed in several reports.[22] Further, it is also reported

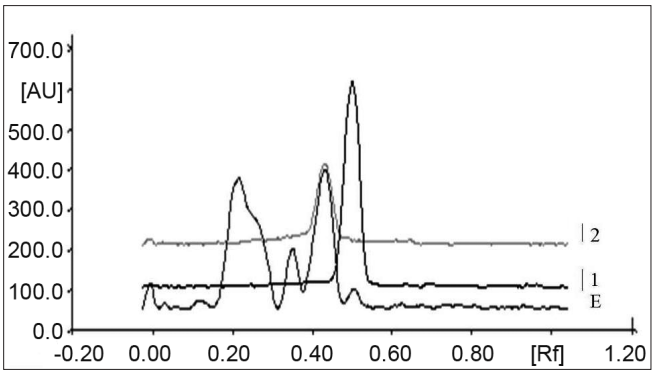

Figure 1: TLC densitometric chromatogram of methanolic extract of green tea with gallic acid standard and catechin standard solution, E: extract; 1: gallic acid; 2: catechin standard solution

Table 2: Effect of administration of doxorubicin alone and along with chronic GTE (30 days) on ECG

Groups	ST interval (msec)	QT interval (msec)	Heart rate (bpm)
I	29.16±1.53	62.5±1.11	403.66±9.51
II	62.5±2.14***	96.66±3.8***	280.83±23.28***
III	50.83±2.71*	84.16±2.71*	336.5±10.57NS
IV	45±2.23***	75.0±3.16***	377.83±23.75**
V	33.33±2.78***	67.5±2.81***	395.83±4.72***
F value	33.39	22.65	9.67
P value	*P* < 0.0001	*P* < 0.0001	*P* < 0.0001

Values are expressed as mean±SEM (n = 6); Group II was compared with Group I; Groups III, IV and V were compared with Group II, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, NS = Non significant

Table 3: Effect of administration of doxorubicin alone and along with chronic GTE (30 days) on serum markers

Groups	LDH (U/l)	CK (U/l)	SGOT (U/ml)
I	169.83±4.62	231.16±12.68	32.33±2.0
II	610.33±77.66***	511.5±17.69***	102.05±5.86***
III	401.33±19.56*	387.0±41.5*	79.65±8.28*
IV	325.5±41.19***	318.16±35.81***	49.26±5.46***
V	307.16±18.04***	261.66±17.77***	41.94±2.35***
F value	15.44	16.48	29.45
P value	*P* < 0.0001	*P* < 0.0001	*P* < 0.0001

Values are expressed as mean±SEM (n = 6); Group II was compared with Group I; Groups III, IV and V compared with Group II, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, NS = Non significant

Table 4: Effect of administration of doxorubicin alone and along with chronic GTE (30 days) on biomarkers of the oxidative stress

Groups	LP (nmoles of MDA/mg protein)	GSH (µg of GSH/mg protein)	SOD (units/mg protein)	CAT (µmoles of H_2O_2 consumed/ minute/mg protein)	Na^+K^+ATPase (µmoles of inorganic phosphorus liberated/minute/ mg protein	Ca^{2+}ATPase (µmoles of inorganic phosphorus liberated/ minute/mg protein	Mg^{2+}ATPase (µmoles of inorganic phosphorus liberated/ minute/mg protein
I	3.06±0.16	9.45±1.21	2.33±0.36	4.02±0.32	7.0±0.2	3.86±0.17	3.01±0.17
II	4.75±0.28***	5.14±0.15***	0.6 ± 0.18**	1.85±0.18***	4.54±0.16***	2.74±0.24**	2.23±0.37^{NS}
III	3.9±0.22*	6.46±0.36^{NS}	1.05±0.25^{NS}	2.03±0.18^{NS}	4.83±0.27^{NS}	2.90±0.075^{NS}	2.78±0.24^{NS}
IV	3.51±0.08***	7.44±0.19^{NS}	1.56±0.23^{NS}	3.21±0.15**	6.10±0.35**	3.02±0.11^{NS}	2.94±0.24^{NS}
V	2.98±0.06***	8.40±0.23**	2.15±0.27**	4.61±0.29***	7.75±0.18***	3.75±0.21**	3.38±0.15*
F value	15.25	8.052	7.210	25.35	31.30	8.36	2.76
P value	*P*<0.0001	*P*=0.0003	*P*=0.00052	*P*<0.0001	*P*<0.0001	*P*=0.0002	*P*=0.0499

Values are expressed as mean ± SEM (n = 6); Group II was compared with Group I; Groups III, IV and V were compared with Group II, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, NS = Non significant

that GTE exhibits more potent antioxidant activity than other conventional antioxidants like vitamins E and C. At the same time, GTE also shows anticancer activity.[10] Thus, GTE could be a better option for ameliorating doxorubicin-induced changes. We conclude that GTE was able to prevent the electrocardiographic abnormalities and pathological changes in biochemical markers, which were induced by doxorubicin. This protection may be due to the catechin content of GTE, which is found be a potent antioxidant than many counterparts.

REFERENCES

1. Doroshow JH. Effect of anthracycline antibiotics on oxygen radical formation in rat heart. Cancer Res 1983;43:460-72.
2. Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 1973;32:302-14.
3. Myers CE, McGuire WP, Liss RH, Ifrim I, Grotzinger K, Young RC. Adriamycin, the role of lipid peroxidation in cardiac toxicity and tumor response. Science 1977;197:165-7.
4. Paracchini L, Jotti A, Bottiroli G, Prosperi E, Supino R, Piccinini F. The spin trap a-phenyl-tert-butyl-nitrone protects against myelotoxicity and cardiotoxicity of adriamycin while preserving the cytotoxic activity. Anticancer Res 1993;13:1607-12.
5. Harbone JB. General procedures and measurement of total phenolics. In: Harbone JB, editor. Methods in Plant Biochemistry. New York: Academic Press; 1996. p. 1-28.
6. Zhu QY, Zhang A, Tsang D, Huang Y, Chen ZY. Stability of green tea catechins. J Agric Food Chem 1997;45:4624-8.
7. Lin AM, Chyi BY, Wu LY, Hwang LS, Ho LT. The antioxidative property of green tea against iron-induced oxidative stress in rat brain. Chin J Physiol 1998;41:189-94.
8. Hiramoto K, Ojima N, Sako K, Kikugawa K. Effect of plant phenolics on the formation of the spin-adduct of hydroxyl radical and the DNA strand breaking by hydroxyl radical. Biol Pharm Bull 1996;19:558-63.
9. Xu Y, Ho CT, Amin SG, Han C, Chung FL. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. Cancer Res 1992;52:3875-9.
10. Ahmad N, Mukhtar H. Green tea polyphenols and cancer: Biologic mechanisms and practical implications. Nutr Rev 1999;57:78-83.
11. Slater TF, Sawyer BC. The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in liver fractions *in vitro*. Biochem J 1971;123:805-14.
12. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay of SOD. J Biol Chem 1972;247:3170-5.
13. Colowick SP, Kaplan NO, Packer L. Methods in Enzymology. Vol. 105. London: Academic Press; 1984. p. 121-5.
14. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
15. Bonting SL. Presence of enzyme system in mammalian tissues. Membrane and Ion Transport. London: Wiley; 1970. p. 257-63.
16. Hjerten S, Pan H. Purification and characterization of two forms of a low affinity Ca2+ATPase from erythrocyte membranes. Biochim Biophys Acta 1983;728:281-8.
17. Ohnishi T, Suzuki T, Suzuki Y, Ozawa K. A comparative study of plasma membrane Mg^{2+}ATPase activities in normal, regenerating and malignant cells. Biochim Biophys Acta 1982;684:67-74.
18. Lowry OH, Rosenbrough NJ, Farr AC, Randell RJ. Protein measurement with folin-phenol reagent. J Biol Chem 1975;193: 265-75.
19. Neri GC, Neri B, Bandinelli M, Tacca MD, Danesi R, Riccardi R. Anthracycline cardiotoxicity *in vivo* and *in vitro* effects on biochemical parameters and heart ultrastructure of the rat. Oncology 1991;48:327-33.
20. Deatley SM, Aksenov MY, Aksenova MV, Harris B, Hadley R, Cole Harper P, *et al*. Anti-oxidants protect against reactive oxygen species associated with adriamycin-treated cardiomyocytes. Cancer Lett 1999;36:41-6.
21. Hrelia S, Bordoni C, Angeloni E, Leoncini T, Gallina T, Lercker G, *et al*. Green tea extracts can counteract the modification of fatty acid composition induced by doxorubicin in cultured cardiomyocytes. Prostaglandins Leukot Essent Fatty Acids 2002;66:519-24.
22. Quiles JL, Huertas JR, Battino M, Mataix J, Ramirez-Tortosa MC. Antioxidant, nutrient and adriamycin toxicity. Toxicology 2000;180:79-95.

Source of Support: Nil, **Conflict of Interest:** None declared.