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Evaluation of Protective Potential of Green Tea on Experimentally Induced Cardiotoxicity and Nephrotoxicity In Rats

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ABSTRACT

Green tea extract (GTE) having so many flavonoid that protect doxorubicin induced cardiotoxicity and nephrotoxity in rats. A single dose of Doxorubicin (20 mg/kg i.p) on 29th day of treatment produced cardiotoxicity. A single dose of cisplatin (6 mg kg^{-1}) was used to induce nephrotoxicity. Wistar Albino rats weighing between 150-200 g were distributed into eight groups comprising of eight animals in each group. Thiobarbituric acid reactive substances (TBARS), GSH, Superoxide dismutase (SOD), and Catalase (CAT) were estimated in heart tissue Reduced glutathione (GSH), Lactate dehydrogenase (LDH), Serum glutamate oxaloacetate (SGOT) and Creatinine phosphokinase (CPK) were estimated in blood. Histopathological studies were performed for the heart tissue of all the groups. DOX induced high serum levels of LDH, CPK and SGOT were reduced while GSH was increased significantly by GTE administration as compared to DOX receiving rats. Pretreatment with GTE ameliorated the cardiac content of GSH, SOD and CAT activities where as MDA level decreased significantly. Scr (serum creatinine) and BUN (blood urea nitrogen) were also estimated. The results support the antioxidant properties of GTE, which indicate cardioprotective role against Doxorubicin (DOX) induced cardiotoxity and nephroprotective role against cisplatin induced nephrotoxicity and its importance as adjuvant therapy in cancer management.

Keywords: Green tea extract, Doxorubicin, cisplatin, Antioxidants, Lipid peroxidation.

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INTRODUCTION

Green Tea is one of the best antioxidant plants having medicinal uses against free radical induced diseases[1]. Green tea contains high levels of polyphenols. Polyphenols from green tea are efficient free radicals and singlet oxygen scavengers that inhibit lipid-peroxidation in in-vitro systems, in experimental animals, and in humans [2]. Green tea has been receiving much attention because it contains useful compounds like polysachharides, flavanoids, vitamin B-complex, vitamin C and fluoride in its natural state. The tea leaf contains as a main component (over 30% of the dry weight) a powerful anti-oxidant, the polyphenol epigallocatechin gallate (EGCG). The chemical formula of EGCG is $C_{22}H_{18}O_{.}$ The leaf also contains the enzyme polyphenol oxidase. Green tea mainly contains poly phenols such as (catechins), flavonols, flavandiols and phenolic acids. The major tea catechins are (-) epigallocatechin-3-gallate (EGCG), (-) epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), (-)-epicatechin (EC) and (-) catechin [4,6,7].

By 2010, CVDs will be the leading cause of death in developing countries. Cardiovascular diseases (CVDs) are responsible for 16.7 million or 29.2% of total global death per year according to world health report 2003. There is a great increase in the incidence of myocardial infarction (MI) [4] in India. More than half of these deaths occurring before the patient reach hospital. The mortality rate with the acute infarction is approx 30%. Although the mortality rate after admission for MI has declined by about 30 % over two decades, approximately 1 out of every 25 patient who survives the initial hospitalization, dies in the first year after M [4].

Myocardial cells get damage due to reactive oxygen species produced during ischemia/reperfusion injury. In both MI and unstable angina, there is increased production of lipid peroxidation and a transient inhibition of protective enzymes such as superoxide dismutase (SOD). Therapeutic intervention that could diminish free radical production or improve impaired anti-oxidant defense mechanism may be one of the treatments of MI [5]. Currently there is an increasing realization that herbs can influence the course of heart disease and its treatment. Recently, several plants of Indian origin have been found to possess anti-oxidant properties and beneficial effects in pathological conditions like atherosclerosis, ischemia, cancer, cataract, and liver dysfunction [3]. This study thus attempts to evaluate the cardioprotective effect of Green Tea (*Camellia sinensis*) Extract (GTE) with reference to Vit. E on Doxorubicin induced cardiomyopathy and cisplatin induced nephrotoxicity in rats.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats weighing (150-200 g) were used for this study. They were housed under ideal laboratory conditions, maintained on standard pellet diet (Lipton rat feed, Ltd; Pune, India) and water *ad libitum* throughout the experimental period The Institutional Animal Ethics Committee Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan, approved the experimental study.

Drugs and chemicals

Doxorubicin was purchased from Sigma Aldrich, USA. Green tea extract (GTE) was obtained from Sanat Products Ltd. India. Vitamin E was obtained from Himedia. All the diagnostic kits were procured from Span diagnostics, Surat and Reckon diagnostics, Baroda, India. All the other chemicals used were of analytical grade.

Experimental design

In this experiment, a total of 64 Wistar Albino rats were used. The rats were divided into eight groups comprising of eight animals in each group. Groups were distributed as follows:

Group I: Normal control rats, received saline (1ml/kg, p.o) for 30 days. Group II: Toxic control rats, received Doxorubicin (20 mg/kg i.p) on 29th day. Group III: GTE control rats, received green tea extract (200 mg/kg/day, p.o.) for 30 days. Group IV: Vitamin E control rats, received Vit. E (100 mg/kg/day, p.o.) for 30 days. Group V: GTE treated-1 rats, received green tea extract (50 mg/kg/day, p.o.) for 30 days + DOX (20 mg/kg i.p.) once on 29th day. Group VI: GTE treated-2 rats, received green tea extract (GTE 100 mg/kg/day, p.o.) for 30 days + DOX (20 mg/kg i.p.) once on 29th day. Group VII: GTE treated-3 rats, received green tea extract (GTE 200 mg/kg/day, p.o. for 30 days + DOX (20 mg/kg i.p.) once on 29th day. Group VIII: Vit. E treated rats, received (Vit. E 100 mg/kg/day, p.o. for 30 days + DOX (20 mg/kg i.p.) once on 29th day.

Doxorubicin in normal saline was administered intraperitonelly (i.p) on 29th day of pretreatment with GTE and estimated after 48 hrs of first dose of DOX. On 31st day blood samples were collected for biochemical estimations. Later the animals were sacrificed and hearts were removed, cleaned and washed with ice-cold saline for biochemical estimations.

In second experiment a total of 24 Wistar Albino rats were used. The rats were divided into three groups comprising of eight animals in each group. Groups were distributed as follows:

Group I: Normal control rats, received saline (1ml/kg, p.o) for 05 days. Group II: Toxic control rats, received Cisplatin (6 mg/kg i.p.) once on 1st day. Group III: GTE (200mg/kg/day, p.o.) once on 1st day before 2 hr of cisplatin administration Cisplatin (6 mg/kg i.p.) once on 1st day. Scr (serum creatinine) and BUN (blood urea nitrogen) were also estimated.

BIOCHEMICAL ESTIMATIONS

Blood GSH estimation

Blood glutathione was assayed by the method of Beutler and Duron (1963)[29]. This method is based on the development of yellow colour when dinitrobenzoic acid (DTNB) is added to compound which reacts with a metaphosphoric acid NaCl-EDTA filtrate of whole blood with a water soluble sulfhydryl reagent group.

Creatinine Phosphokinase (CPK) estimation

CPK was estimated by the method of Rosalki (1967) [30], in this reaction creatine kinase (CK) catalyses the formation of ATP from creatine phosphate and ADP. Glucose is converted to Glucose-6-phosphate by hexokinase using ATP as source of phosphate moiety. Glucose-6-phosphate is oxidized by Glucose-6-phosphate dehydrogenase to 6-phosphogluconate reducing NADH to NADPH. The reaction after the lag phase is monitored by the increase in absorbance at 340 nm and is directly proportional to the creatine kinase activity (i.e. the formation of NADPH is in equimolar amount as that of formation of creatine).

Lactate Dehydrogenase (LDH) estimation

The activity of lactate dehydrogenase was assayed by the method of Lum and Gambino (1974)[31]. LDH catalyses the conversion of lactate to pyruvate and the amount of pyruvate formed are measured at 340 nm.

Serum glutamate oxaloacetate transaminase (SGOT) estimation.

SGOT was estimated by the method of Reitman and Frankel (1957) [30]. In this method Aspartate transaminase (AST) catalyses the transfer of amino group from L-Aspartate to α-ketoglutarate with the formation of oxaloacetate and glutamate, Oxaloacetate so formed is coupled with 2,4-dinitrophenylhydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and this is measured colorimetrically at 505 nm.

Post-mitochondrial supernatant preparation (PMS) [32]

Heart were removed quickly, perfused immediately with ice cold normal saline and homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing potassium chloride (1.17%) using a Potter Elvehjem homogenizer. The homogenate was centrifuged at 800 × g for 5 min at 4^{o}C in a refrigerated centrifuge to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 × g for 20 min at 4^{o}C to get the PMS which was used to assay GSH, TBARS, SOD and CAT activity.

Determination of GSH

GSH content was estimated by method of Sedlak and Lindsay (1968)[33]. The tissues were homogenized in 0.02M EDTA. Aliquots of 5 ml of the homogenates were mixed in test tube with 4 ml of cold distilled water and 1 ml of 50% TCA. The tubes were shaken for 10 minutes using

vortex mixer and the centrifuged at 1200 × g for 15 minutes. Following centrifugation 2 ml of supernatant was mixed with 4 ml of 0.4 M tris buffer (pH–8.9). The whole solution was mixed and 0.1 ml of 0.01 M DTNB {5, 5'-Dithiobis (2-nitrobenzoic acid)} was added to it. The absorbance was read within 5 minutes of addition of DTNB at 412 nm using UV-spectrophotometer (Shimadzu, UV-1601, Japan) against a reagent blank with no homogenate.

Determination of LPO

LPO was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids (Ohkawa et al., 1979) [34]. Tissues were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) that contained KCl (1.17% w/v), using motor driven Teflon pestle. Aliquot of 1 ml of the suspension medium was taken from the supernatant obtained after the centrifugation of tissue homogenate (10% w/v) at 10,500 × g. 0.5 ml of 30% TCA followed by 0.5 ml of 0.8% TBA was then added to it. The tubes were kept in shaking water bath for 30 minutes at 80^{o}C. After 30 minutes of incubation tubes were taken out and kept in ice cold water for 10 minutes. These were then centrifuged at 800 × g for 15 minutes. The absorbance of supernatant was read at 540 nm at room temperature against appropriate blank. The concentration of MDA was measured from the standard calibration curve prepared by using tetraethoxypropane. Protein was estimated by the method of Lowry et al. (1951). Lipid peroxidation was expressed as n moles of MDA per milligram of protein.

Determination of SOD activity

SOD activity was measured according to the method of Marklund (1974) [35]. The enzyme activity was expressed as units mg^{-1} protein and one unit of enzyme is defined as the enzyme activity that inhibits autoxidation of pyrogallol by 50%.

Determination of CAT activity

CAT activity was estimated following the method of Claiborne (1985) [36]. The reaction mixture consisted of 1.95 ml phosphate buffer (0.1 M, pH 7.4), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml PMS (10% w/v) in a final volume of 3 ml. Changes in absorbance were recorded at 240 nm. The enzyme activity was calculated as n moles of H_2O_2 consumed min^{-1} mg^{-1} protein.

Statistical analysis

Data were expressed as the mean ± standard error (S.E) of the means. For a statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The Tukey-Karmer test post hoc was applied to identify significance among groups. $P<0.05$ was considered to be statistically significant. Graph Pad software, Inc. (version 3.06) was used for statistical analysis.

RESULTS AND DISCUSSION

Effect of Green tea extract on Doxorubicin-induced blood GSH levels

The blood GSH levels of Toxic control, i.e., Doxorubicin treated group(Group II) showed a significant decrease when compared with Normal control(Group I) (P<0.001). GTE 50 mg/kg + DOX treated group (Group V) did not show any significant increase when compared with Group II. GTE 100 mg/kg + DOX, GTE 200 mg/kg + DOX and Vitamin E 100 mg/kg + DOX (Group VI, VII & VIII, respectively) showed a significant increase in blood GSH levels when compared with Group II (P<0.001). Also, Group VII showed a significant increase in blood GSH levels when compared with Group VI (P<0.001). GTE 200 mg/kg only(Group III) and Vitamin E100 mg/kg only(Group IV) treated groups however showed the same value as that of the group I

Effect of Green tea extract on Doxorubicin-induced LDH activity

The LDH activity in Group II rats were found to be significantly high when compared with Group I rats (P<0.001). Group V (P<0.01) and Group VI, VII, VIII (P<0.001) showed a significant decrease in LDH activity when compared with group II. Group VI showed a significant decrease in LDH activity when compared with group V (P<0.001). Group VII showed a significant decrease in LDH activity when compared with Group VI (P<0.001). Per se groups (group III and IV) however showed no significant change when compared with Group I.

Effect of Green tea extract on Doxorubicin-induced CPK activity

The CPK activity in Group II rats were found to be significantly high when compared with Group I (P<0.001). Group V (P<0.05) and Groups VI, VII, VIII (P<0.001) showed a significant decrease in CPK activity when compared with Group II. Group VI showed a significant decrease in CPK activity when compared with Group V (P<0.001). Group VII showed a significant decrease in CPK activity when compared with Group VI (P<0.001).The test drug only treated Groups (III and IV) showed the same activity as found in Group I.

Effect of Green tea extract on Doxorubicin-induced SGOT level

The enzyme showed a significant increase in its activity in Group II when compared with Group I (P<0.001). Group V showed a significant decrease in SGOT activity when compared with Group II (P<0.01). Group VI, VII and VIII showed significant decrease in SGOT activity when compared with Group II (P<0.001).group VI did not show significant decrease when compared with Group V. Group VII however showed a decrease in its activity when compared with Group VI (P<0.001). Test drug only treated groups (Group III and IV) showed the same activity when compared with Group I.

Effect of Green tea extracts on Doxorubicin-induced tissue GSH levels

The tissue GSH levels of Group II showed a significant decrease when compared with Group I (P<0.001). Group V (P<0.01) and Group VI, VII, VII (P<0.001) showed a significant increase in tissue GSH levels when compared with Group II. Group VI showed a significant increase in tissue GSH levels when compared with Group V (P<0.001). Group VII showed a significant increase in tissue GSH levels when compared with Group VI (P<0.001). Per se Groups showed the same level as that of Group I.

Effect of Green tea extracts on Doxorubicin-induced tissue TBARS levels

The TBARS concentration of Group II showed a significant increase when compared with Group I (P<0.001). Group V (P<0.05), Group VI (P<0.01) and Group VII (P<0.001) showed a significant decrease in TBARS concentration when compared with Group II. Group VI did not show any significant decrease in TBARS concentration when compared with Group V, but Group VII showed a significant decrease when compared with Group VI (P<0.001). The test drugs only treated groups however showed the same level as that of Group I.

Effect of Green tea extract on Doxorubicin-induced tissue SOD activity

The SOD activity showed a significant decrease in Group II when compared with Group I (P<0.001). Group V (P<0.01) and Group VI, VII.VIII (P<0.001) showed a significant increase in SOD activity when compared with Group II. Group VI did not show any significant increase in SOD activity when compared with Group V, however, Group VII showed a significant increase in SOD activity when compared with Group VI (P<0.001). Per se group showed the same levels when compared with Group I.

Effect of Green tea extract on Doxorubicin-induced tissue CAT activity

Group II showed a significant increase in CAT activity when compared with Group I (P<0.001). Group V (P<0.05) and Group VI, VII, VIII (P<0.001) showed a significant increase in CAT activity when compared with Group II. There was no significant increase in CAT activity in Group VI when compared with Group V, but Group VII showed a significant increase in CAT activity when compared with Group VI (P<0.001) per se however showed the same activity when compared with Group I.

Effect of Green tea extract on Cisplatin-induced BUN and Scr activity

Group II of cisplatin study showed increase in BUN and Scr activity and Group III showed significant decrease in BUN and Scr activity showing protective effect of Green tea extract.

Effect of green tea extract on Doxorubicin-induced histological changes on heart

Figure 1-8 shows the histopathological observation.

Figure 1 shows Group I (Normal saline treated group): Section showing normal myocardium.

Figure 2 shows Group II (Doxorubicin treated group): Section showing interstitial polymorphonuclear infiltrate, edema and focal myocardial necrosis.

Figure 3 shows Group III (GTE 200 mg/kg, per se): Section showing morphologically normal myocardium.

Figure 4 shows Group IV (Vit. E 100mg/kg, per se): Section showing morphologically normal myocardium.

Figure 5 shows Group V (GTE 50 mg/kg + Doxorubicin): Section showing myocardial necrosis, disruption of architecture, interstitial edema and inflammatory cell infiltrate.

Figure 6 shows Group VI (GTE 100 mg/kg + Doxorubicin): Section showing mild interstitial edema and moderate amount of inflammatory cell infiltrate mild degeneration of myocardium.

Figure 7 shows Group VII (GTE 200mg/kg + Doxorubicin): Section showing myocardium of nearly normal appearance with very mild inflammatory cell infiltrate and absence of necrosis.

Figure 8 shows Group VIII (Vit. E 100mg/kg + Doxorubicin): Section showing presence of moderate amount of interstitial inflammatory cell infiltrate, focal necrosis and edema.

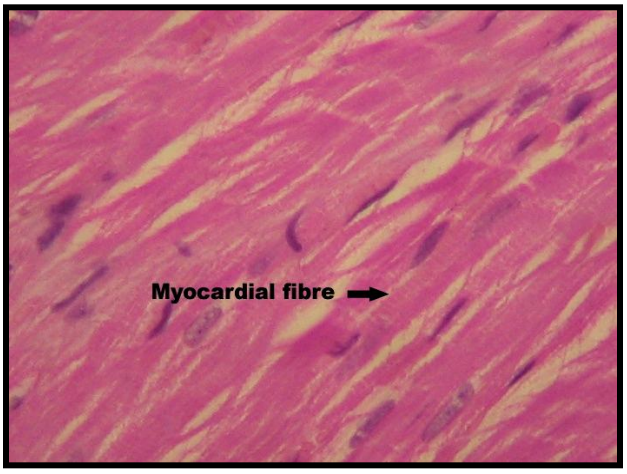

Figure. 1: High Power photomicrograph of the heart of normal control (Group I) showing normal myocardial fibres. There is no vaculation, necrosis or inflammation seen (HE x 400).

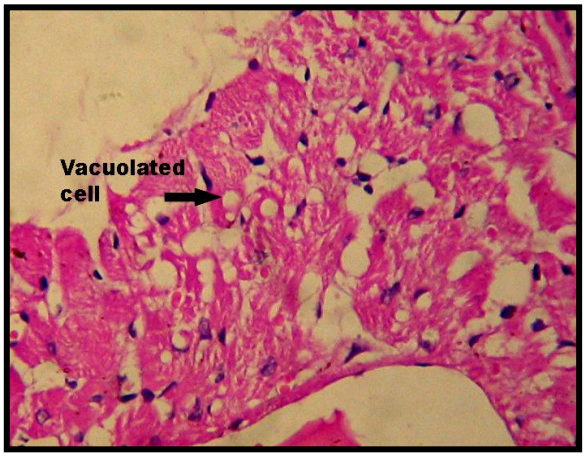

Figure. 2: High Power photomicrograph of the heart of DOX-treated (Group II) animal showing myocardium fibres with extensive small and large cytoplasmic vacuoles (HE x 400).

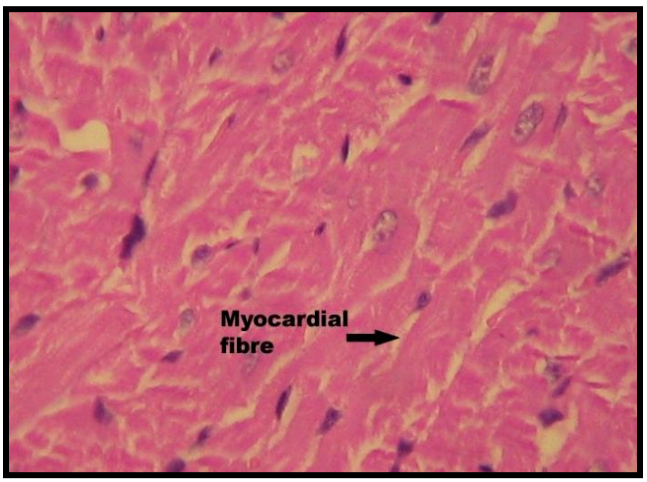

Figure. 3: High power photomicrograph from animal treated with GTE (200 mg/kg) *per se* (Group III) showing normal appearance of cardiac muscle fibres (HE x 400).

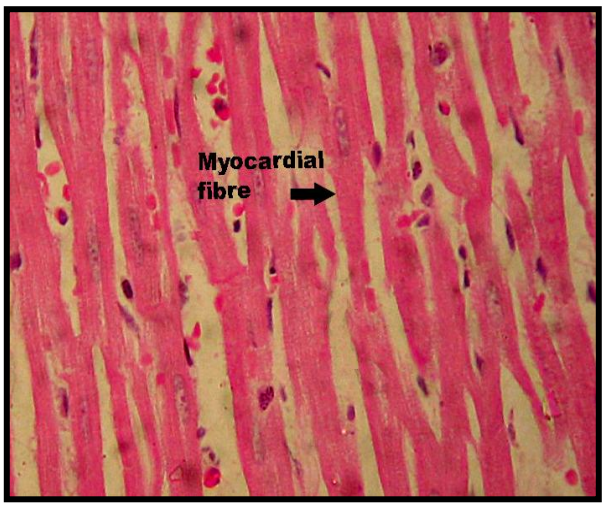

Figure. 4: High power photomicrograph from animal treated with Vitamin E *per se* (Group IV) has shown normal appearance of cardiac muscle fibres (HE x 400).

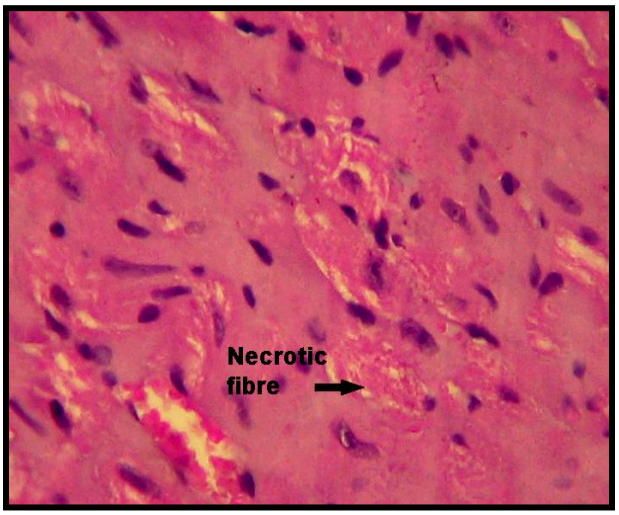

Figure. 5: High power photomicrograph from animal pretreated with GTE (50 mg/kg) followed by DOX (Group V) showing a few necrotic myocardial cells (HE x 400).

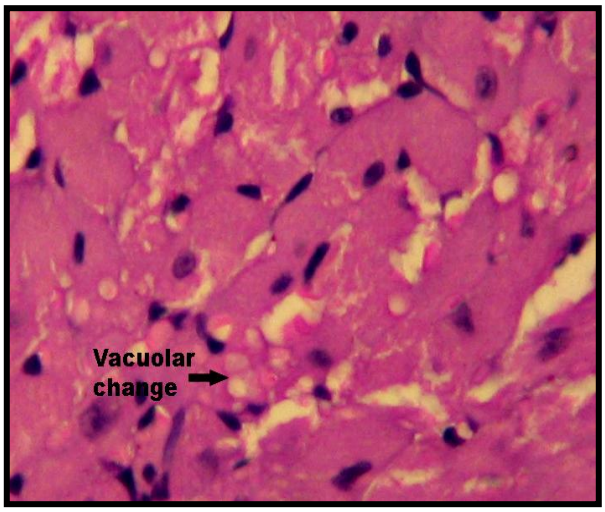

Figure. 6: High power photomicrograph from animal treated with 100 mg GTE and DOX (Group VI) showing cardiac muscle fibre of normal shape, size and configuration. A single myocardial fibre with intra cytoplasmic vacuoles is seen in the photograph (HE x 400).

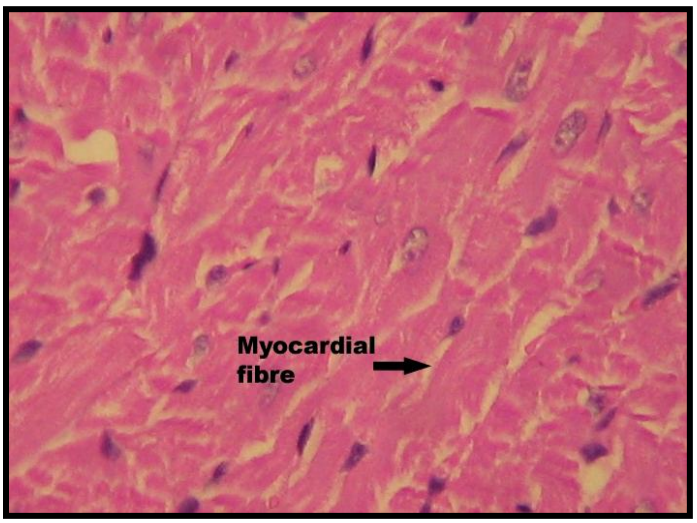

Figure. 7: High Power photomicrograph of the heart of BTE (200 mg/kg) group (Group VII) showing normal myocardial fibres. There is no vaculation, necrosis or inflammation seen (HE x 400).

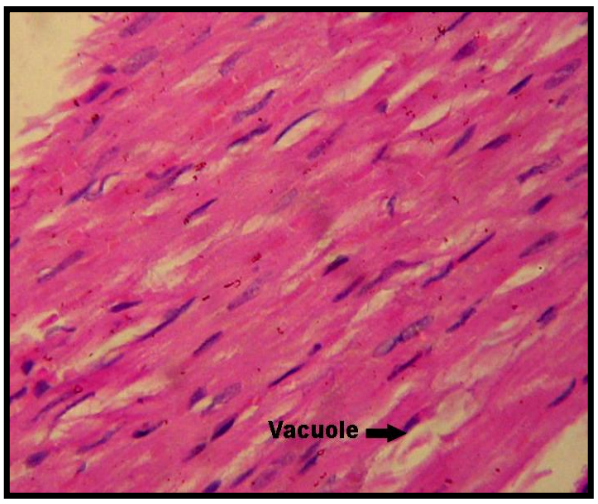

Figure. 8: High power photomicrograph from animal treated with Vitamin E and DOX (Group VIII) showing cardiac muscle fibres of normal shape, size and configuration with minimal disorganization of myocardial fibre (HE x 400).

Table 1: Effect of green tea extract (GTE) on blood glutathione (GSH), lactate dehydrogenase (LDH), Creatinine phosphokinase (CPK), and Serum glutamate oxaloacetate (SGOT) in normal and Doxorubicin (DOX) induced myocardial infarction in rats.

Group	Treatment	Blood GSH	LDH (IU/L)	CPK (IU/L)	SGOT (IU/ml)
I	Normal control	2.07 ± 0.03	422.63±19.90	142.29± 5.81	98.50 ± 4.75
II	Doxorubicin (20 mg/kg i.p.)	1.04 ± 0.04	986.27±25.21[a]	297.68±7.97[a]	250.00 ±6.59 [a]
III	GTE (200mg/kg/day, p.o.), per se	2.18 ± 0.05	414.58± 18.70	134.66±4.97	99.2900 ±3.38
IV	Vit. E (100mg/kg/day, p.o.), per se	2.12± 0.06	421.08 ±17.54	139.85±5.37	102.75± 3.37
V	GTE (50mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	1.11 ± 0.04	954.42± 20.83 **	292.13±6.53 *	242.75± 4.26 **
VI	GTE (100mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	1.09 ±0.03 *** #	895.63 ±23.33*** ##	275.63±7.25*** ##	235.75± 4.06 ***
VII	GTE (200mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	1.46 ±0.06 *** ## b	611.45± 24.36*** ## b	221.49±4.54*** ## b	202.50± 7.23 *** ## b
VIII	VitaminE (100mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	1.35 ±0.03 ***	629.57± 22.95***	230.22±5.17***	231.00± 5.12 ***

a : ($p<0.001$) when compared to Group I. * : ($p<0.05$), ** : ($p<0.01$), *** : ($p<0.001$) when compared to Group II.

b : ($p<0.001$) when compared to group VI. # : ($p<0.01$), ## : ($p<0.001$) when compared to Group V.

1. Each group contains eight animals. 2. Data shown in mean ± SD. 3. Data is analyzed with ANOVA followed by LSD.

Table 2: Effect of green tea extract (GTE) on heart glutathione (GSH), Thiobarbituric acid reactive substances (TBARS), Superoxide dismutase (SOD), and Catalase (CAT) in normal and Doxorubicin (DOX) induced myocardial infarction in rats.

Group	Treatment	TISSUE GSH (ìg/g wet wt. tissue)	TBARS (çmoles MDA/mg protein)	SOD (U/mg protein)	CAT (çmoles of H_2O_2 consumed/min /mg protein)
I	Normal control	578.37 ± 8.97	2.10 ± 0.07	1.93 ± 0.07	8.00 ± 0.20
II	Doxorubicin (20 mg/kg i.p.)	331.13 ± 10.88 [a]	4.41 ± 0.11 [a]	0.98 ± 0.05 [a]	250.00 ±6.59 [a]
III	GTE (200mg/kg/day, p.o.), per se	2.18 ± 0.05	2.02 ± 0.06	2.01 ± 0.10	8.80 ± 0.23
IV	Vit E (100mg/kg/day, p.o.), per se	580.36 ± 12.61	2.08 ± 0.07	1.97 ± 0.11	100.75± 3.37
V	GTE (50mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	366.36 ± 8.12 [***] [#]	4.24 ± 0.06 [**]	1.17 ± 0.04 [***]	5.64 ± 0.12 [***]
VI	GTE (100mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	636.36 ± 8.12 [***]	4.24 ± 0.06 [**]	1.17 ± 0.04 [***]	5.64 ± 0.12 [***]
VII	GTE (200mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	477.38 ± 11.35 [***] [##] [b]	2.81 ± 0.08 [***] [##] [b]	1.53 ± 0.06 [***] [##] [b]	7.50 ± 0.14 [***] [##] [b]
VIII	Vit. E (100mg/kg/day, p.o.) + Doxorubicin (20 mg/kg i.p.)	434.52 ± 7.64 [***]	3.12 ± 0.10 [***]	1.52 ± 0.07 [***]	7.02 ± 0.16 [***]

a : ($p<0.001$) when compared to Group I. *: ($p<0.05$), ** : ($p<0.01$), *** : ($p<0.001$) when compared to Group II.

b : ($p<0.001$) when compared to group VI. #: ($p<0.01$), ##: ($p<0.001$) when compared to Group V.

1 Each group contains eight animals. 2. Data shown in mean ± SD. 3. Data is analyzed with ANOVA followed by LSD.

Table 3: Effect of green tea extract (GTE) on BUN and Scr activity cisplatin induced nephrotoxicity in rats.

Group	Treatment	BUN (mg dl^{-1})	Scr (mg dl^{-1})
1	Vehicle	18.02 ± 1.60[***]	0.73 ± 0.03[**]
2	Cisplatin	57.50 ± 2.44	1.40 ± 0.30
3	Cisplatin + GTE (2 h before cisplatin)	32.30 ± 1.51[**]	1.14 ± 0.05[*]

n = 5, mean ± SEM;
$^{*}P < 0.5$, $^{**}P < 0.01$, $^{***}P < 0.001$ significantly different compared with cisplatin-treated group.

It has been proposed that catechin polyphenols reacts with peroxy radicals involving termination of radical chain reaction [12,13]. In a variety of in-vitro and in-vivo studies green tea polyphenols were found to scavenge NO, H_2O_2, $^{\cdot}OH^{-}$ and $O_2^{\cdot -}$ and reduce oxygen free radical damage [4,8-11]. Tea polyphenol act as anti-oxidant in-vitro by scavenging reactive oxygen and nitrogen species and chelating redox-reactive transition metal ion. The reduction potential of the polyphenolic anti-oxidants through the extended conjugation and the increasing number of hydroxyl group define their free radical scavenging activities [12].

The fact that, catechins are rapidly and extensively metabolized during absorption in the small intestine, colon and liver, emphasizes the importance of demonstrating their anti-oxidant activity in-vivo [14-16]. Tea flavonoids are potent anti-oxidant that is absorbed from the gut after consumption. The consumption consistently leads to a significant increase in the anti-oxidant capacity of the blood[16,17]. Most promising are the consistent findings in animal models of skin, lung, colon, liver and pancreatic cancer, that tea and tea polyphenol administration inhibits carcinogen induced increase in the oxidized DNA base, 8-hydroxy-2-deoxyguanosine.

The scientific support is the strongest for the protection of DNA from oxidative damage after green tea consumption [16]. In animal model of atherosclerosis, green tea administration has resulted in modest improvement in the resistance of lipoproteins to ex vivo oxidation [15]. One mechanism that might explain a beneficial effect of tea on the cardiovascular system is that it improves the vascular endothelium function [18]. EGCG is considered a potent anti-oxidant and major anti-carcinogenic component in tea [4]. In fact, it was reported that pre-treatment with green tea extract increased the level of CAT activity in the lung, liver and kidney [19].

Doxorubicin induces myocardial necrosis, which is maximal in the sub endothelial region of the left ventricle and interventricular septum. These changes resemble the sub endothelial necrosis produced by myocardial necrosis produced in human [20].

Doxorubicin, a synthetic catecholamine, has cardiotoxic effect on the myocardium. Amongst the various mechanisms proposed to explain Doxorubicin induced cardiac damage, generation of highly cytotoxic free radicals through auto oxidation of catecholamine has been implicated as one of the most causative factor. This free radical mediated peroxidation of membrane phospholipids and consequent changes in membrane permeability as well as intracellular Ca^{2+} overload is the primary target responsible for cardiotoxicity induced by Doxorubicin [21-23].

Free radical scavenging enzyme such as CAT, SOD, GPx and glutathione-S- transferase are the first line cellular defence against oxidative injury, decomposing O_2^- and H_2O_2 before their interaction to form the more reactive hydroxyl radical ($^{\cdot}OH^-$). The equilibrium between these enzymes is an important process for the effective removal of oxidative stress in intracellular organelles. The second line of defence consist of the non enzymatic scavengers viz., ascorbic acid , α- tocopherol, ceruloplasmin and sulfhydril containing compounds, which scavenge residual free radicals escaping decomposition by the antioxidant enzyme [24].

An increase in the activities of marker enzymes (CPK, LDH, and SGOT) in the serum on treatment with Doxorubicin could be due to the leakage of enzymes from heart as a result of necrosis and the amount of enzymes appearing in serum is in proportion to the number of

necrotic cells [25,26]. In this case we also have observed an increase in the activities of CPK, LDH, and SGOT in serum after Doxorubicin administration.

In this study, Doxorubicin administration is found to reduce the GSH content of heart and of blood. It also reduces the antioxidant enzyme (SOD and CAT) activity in cardiac tissue, which is well in accordance with the earlier finding [24,27]. The cytotoxic free radicals generated by Doxorubicin, cause the loss of membrane integrity by initiating the lipid peroxidation of membrane bound polyunsaturated fatty acids [20]. In this case we also have observed an increase in the levels of TBARS in the heart tissue after Doxorubicin administration.

Chronic oral administration of GTE in all the doses prevented Doxorubicin induced myocardial injury at different levels. Myocardial activity of antioxidant enzyme (SOD and CAT) shown to be preserved in all the three doses but the activity was more significant in dose 200 mg/kg when compared to 50 mg/kg groups. GTE at doses 100 and 200 mg/kg were shown to increase the myocardial and blood GSH levels, while GTE at dose 50 mg/kg increased only myocardial GSH levels, hence GTE at doses 100 and 200 mg/kg showing significant protection in terms of preservation of endogenous antioxidant system. The increase in endogenous antioxidant activity leads to decrease in lipid peroxidation of membrane phospholipids which is shown in this study by reduced myocardial TBARS concentration in all the three doses of GTE treated groups. GTE at a dose of 200 mg/kg showed significant inhibitory effect on lipid peroxidation when compared to groups treated with the dose 50 and 100 mg/kg. A significant reduction in the activities of marker enzymes LDH, CPK, SGOT) in serum is indicative of the fact that green tea has cardioprotective action and maintains membrane integrity of myocytes and significant decrease in the amount of BUN and Scr in blood shows nephroprotective effect.

The result of histopathology further confirms the Doxorubicin induced myocardial damage. The DOX treated group (Group II, Figure. 2) demonstrated interstitial polymorphonuclear infiltrate, edema and focal myocardial necrosis as compared to normal control group (Group I). The treatment with GTE 50 mg/kg (Group V, Figure.5) did not demonstrate the reversal of myonecrosis, edema and inflammatory cell infiltration, which have been seen with DOX, treated group (Group II). However, the treatment with GTE 100 mg/kg (GroupVI, Figure. 6) and GTE 200 mg/kg (Group VII, Figure. 7) groups did demonstrate the reversal of the conditions, which were seen with DOX, treated group (Group II). Furthermore, between these two doses GTE 200 mg/kg treated group (Group VII) have shown better restoration or preservation of myocardium. Vitamin E 100 mg/kg treated group (Group III, Figure.3) have only demonstrated mild reversal of myonecrosis, edema and inflammatory cell infiltration as seen with DOX treated group

(Group II). The only test drugs treated groups, ie., Group III (Figure. 3) and Group IV (Figure. 4), however, have shown the same myocardial status as that of the control. Thus, the histopathological results show myocardial damage by doxorubicin and its prevention by GTE.

In the present study chronic oral administration of Vit. E, taken as reference drug, attenuated the increase in TBARS level in myocardium upon Doxorubicin administration. α- tocopherol, being a lipid soluble chain breaking antioxidant reacts with O_2^- and lipid peroxy radicals, thereby inhibiting lipid peroxidation. Vitamin E increased the myocardial antioxidant enzymes (SOD and CAT) and GSH levels in myocardium as well as in blood. The significant reduction in activities of marker enzymes (LDH, CPK, SGOT) in serum in Vitamin E treated group suggests that it has cardioprotective action and maintain the membrane integrity of myocytes. The cardioprotective effect of Vit. E is well known in Doxorubicin induced myocardial infarction and the results obtained in this study are consistent with the earlier findings [28].

CONCLUSION

The results of this study based on biochemical and histopathological observations, indicate the protective role of GTE against doxorubicin induced MI and cisplatin induced nephrotoxicity. Although the protection is seen with all the three doses, i.e., 50 mg/kg, 100 mg/kg and 200 mg/kg body weight, the best protective effect is indicated by 200 mg/kg dose.

REFERENCES

1. Ebadi M, Green and Black teas. Pharmacodynamic basis of Herbal medicine. CRC. Press. Boca. Raton 2001; 435-38.
2. Zhong Z, Froh M, Connor HD, Li X, Conzelmann LO, Mason RP, Lemasters JJ, Thurman RG. Prevention of hepatic ischemia - reperfusion injury by Green tea extract. Am J Physiol Gastrointest Liver Physiol 2002; 283: 957-64.
3. Sharma M, Kishore K, Gupta SK, Joshi S, Arya DS. Cardioprotective potential of Ocimum sanctum in Doxorubicin induced myocardial infarction in rats. Mol. Cell. Biochem 2001; 225:75-83.
4. Antman EM, Braunwald E. Acute myocardial infarction. In : Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. (Eds.), Harrisonn's Principles of Internal Medicines., Ed. 15, Vol.1. Mc Graw-Hill Education, Asia; 2003. 1386-99.
5. Gupta SK, Mohanty I, Talwar KK, Dinda A, Joshi S, Bansal P, Saxena A, Arya DS. Cardioprotection from ischemia and reperfusion injury by Withania somnifera : A haemodynamic, biochemical and histopathological assessment. Mol. Cell. Biochem 2004;

260:39-47.

6. Kim JI, Hong SB, Row KH. Effect of particle size in preparative reversed phase high performance liquid chromatography on the isolation of epigallocatechin gallate from Korean green tea. J Chromatogra A 2002; 949: 275- 80.
7. Weisburger JH. Tea and Health: The Underlying Mechanism. P.S.E.B.M 1999; 220:271-75.
8. Robbers JE, Tyler VE, (Eds). Performance and Immune Deficiencies. In: Tyler's Herbs of choice. CBS Publishers and Distributors, New Delhi: 2002. 235-60.
9. Nakagawa T, Yokozawa T. Direct scavenging of nitric oxide and Superoxide by green tea. Fd Chem Toxicol 2002; 40:1745-50.
10. Xu ZJ, Yeung SYV, Chang Q, Huang Y, Chen ZY. Comparison of antioxidant activity and bioavailability of tea epicatechins with their epimers. Br J Nutr 2004; 91:873-81.
11. Buttemeter R, Philipp AW, Schlenzka L, Mall JW, Beissenhirtz M, Lisdat F. Epigallocatechin Gallate can significantly decrease free oxygen radical in the Reperfusion injury In Vivo. Trans Proc 2003; 35:3116-20.
12. Panala AS, Rice Evans CA, Halliwell B, Singh S. Inhibition of Peroxynitrite Mediated Tyrosine Nitration by Catechin Polyphenols. Biochem Biophys Res Communis 1997; 32:164-68.
13. Guleria RS, Jain A, Tiwari V, Misra MK. Protective effect of green tea extract against the erythrocytic oxidative stress injury during Mycobacterium tuberculosis infection in mice. Mol Cell Biochem 2002; 236:173-81.
14. 14 Spencer JPE. Metabolism of Tea Flavanoid in the Gastrointestinal Tract. J Nutr 2003; 133:3255-61.
15. Frei B, Higdon JV. Antioxidant Activity of Tea Polyphenols In Vivo: Evidence from Animal Studies. J Nutr 2003; 133:3275-84.
16. Rietveld A, Wiseman S. Antioxidant Effect of Tea: Evidence from Human Clinical Trials. J Nutr 2003; 133:3285-92.
17. Leenen R, Roodenburg AJC, Tijburg BM, Wiseman SA. A single dose of tea with or without milk increases plasma antioxidant activity in humans. Eur J Clin Nutr 2000; 54: 87-92
18. Vita JA. Tea Consumption and Cardiovascular Disease: Effect on Endothelial Function. J Nutr 2003; 133: 3293-97.

19. Hong JT, Ryu SR, Kim HJ, Lee SH, Yun YP, Lee BM, Kim PY. Protective effect of green tea extract on ischemia/reperfusion induced brain injury in Mongolian gerbils. Brain Res 2001; 888:11-18.
20. Noronha Dutra AA, Steen EM, Woolf N. The Early changes induced by Doxorubicin in the endocardium and adjacent Myocardium. Am J Pathol 1984; 114:231-39.
21. Singal PK, Kapur N, Dhilon KS, Beamish RE, Dhalla NS. Role of free radical in catecholamine-induced cardiomyopathy. Can J Physiol Pharmacol 1982; 60: 1390-97.
22. Rona G, Chappel CL, Balazs T, Gaudry R. An Infarct like Myocardial lesion and other toxic manifestation produced by Doxorubicin in rat. Arch Pathol 1959; 67: 443-55.
23. Behonick GS, Novak MJ, Nealley EW, Baskin SI, Toxicology Updat. The cardiotoxicity of the Oxidative stress metabolites of catecholamines (Aminochromes). J Appl Toxicol 2001; 21:S15-S22.
24. Nirmala C, Puvanakrishna R. Protective role of curcumin against Doxorubicin induced myocardial infarction in rats. Mol Cell Biochem 1996; 159:85-93.
25. Ithayarasi PA, Padmavathy VN, Shyamala Devi CS. Effect of α- tocopherol on Doxorubicin induced myocardial infarction in rats. Biochemical and histological evidences. Indian J Physiol Pharmacol 1996; 40:297-302.
26. Geetha A, Sankar R, Thankamani M, Shyamal Devi CS. α-tocopherol reduces Doxorubicin induced toxicity in rats. Biochemical and histological evidences. Indian. J Physiol Pharmacol 1990; 34:94-100.
27. Mohanty I, Arya DS, Dinda A, Talwar KK, Joshi S, Gupta SK. Mechanism of cardioprotective effect of Withania somnifera in experimentally induced Myocardial Infarction. Basic Clin Pharmacol Toxicol 2004; 94:184-90.
28. Lum G, Gambino SR. A comparison of serum Vs heparinized plasma for routine chemistry test. Am J Clin Pathol 1974; 61:108-13
29. Beutler, E., Duron, O., Kelly, B.M.,. Improved method for the determination of
30. blood glutathione. J. Lab. Clin. Med 1963;61, 882-888.
31. Reitman, S., Frankel, S., 1957. A calorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminases. Am. J. Clin. Pathol; 28, 56-63.
32. Rosalki, S.B.,. An improved procedure for serum creatine phosphokinase determination. J Lab Clin Med. 1967;69(4):696-705.

33. Lora E Rikans, K. Roger Hornbrook, Lipid peroxidation, antioxidant protection and aging, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease, Issues 1997, 1362(2‑3): 116–127.
34. Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein bound and nonprotein sulfhydril group in tissue with Ellman's reagent. Anal. Biochem. 25, 192- 205.
35. Okhawa, H., Oshishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem; 95, 351-358.
36. Marklund, S., Marklund, G., 1974. Involvement of superoxide anion radical in autooxidation by pyrogallol and a convenient assay of superoxide dismutase. Eur. J. Biochem; 47, 469- 474.
37. Caliborne, A.L., 1985. Assay of catalase. In: Greenworld RA (Ed.), Handbook of methods of oxygen free radical research. CRC press, Boca Raton, pp; 283- 284.