

Combined Effect of Green Tea Extract and Vitamin E on Serum and Heart Tissue Lipids, Lipid Metabolizing Enzymes and Histopathological Alteration in Isoproterenol-Induced Myocardial Infarction in Rats

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Abstract

The present study investigated the protective effect of green tea and vitamin E combination on serum and heart tissue lipid profile, lipid metabolizing enzymes and histopathological changes in isoproterenol (ISO)-induced myocardial infarction in rats. Adult male albino rats, treated with ISO (200mg/kg, s.c.) for 2 consecutive days at an interval of 24 hrs. showed a significant increase in the levels of triglycerides (TG), total cholesterol (TC) and free fatty acids (FFA), in both serum and cardiac tissue. A rise in the levels of phospholipids (PL), low density lipoprotein (LDL) and very low density lipoprotein-cholesterol (VLDL-c) was also observed in the serum of isoproterenol-intoxicated rats. Further, a decrease in the level of high density lipoprotein-cholesterol (HDL-c) in serum and phospholipid levels, in the heart of isoproterenol-intoxicated rats was observed. Further a significant decrease in the activities of cholesterol ester synthetase (CES) and lecithin: cholesterol acyl transferase (LCAT) was shown whereas lipoprotein lipase (LPL) was found to be increased. Administration of alcoholic extract (60% polyphenols) of green tea (100 mg/kg/day, p.o.) and

Vitamin E (DL- α -Tocopherol acetate) (100 mg/kg/day, p.o.) together for 30 consecutive days and challenged with ISO on day 29th and 30th, significantly attenuated these alterations and restored the levels of serum and heart lipids along with lipid metabolizing enzymes. Histopathological observations were also in correlation with the biochemical parameters. These findings indicate the protective effect of green tea and vitamin E in combination during ISO-induced myocardial infarction in rats.

Keywords

Green tea • Lipid profile • Vitamin E • Isoproterenol • Histopathology

Introduction

Cardiovascular disease (CVD) remains the principal cause of death in both developed and developing countries. Studies have shown that high levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c) and apolipoproteins A-I and low levels of high density lipoproteins cholesterol (HDL-c) are the risk factors of CVD [1, 2]. Reactive oxygen species may contribute to the events of atherogenesis and leading to the progression of atherogenic lesions by promoting oxidation of low density lipoproteins [3]. Isoproterenol has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of heart muscles. It also increases the level of serum and myocardial lipids [4] and also increases the level of LDL cholesterol in the blood which in turn leads to coronary heart disease [5].

Green tea is an excellent source of polyphenol antioxidants, and mainly contains catechins especially (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin gallate (ECG) and (–)-epigallocatechin gallate (EGCG) [6]. Catechins have beneficial effects in prevention of cardiovascular diseases including LDL oxidative susceptibility, serum lipids and lipoprotein concentration [7]. Green tea reduces doxorubicin induced cardiotoxicity, cyclosporine A-induced oxidative damage, chemical induced lung tumorigenesis and iron induced lipid peroxidation in brain homogenate [8–11]. Vitamin E is a chain breaking lipid soluble antioxidant has been shown to slow or inhibit the oxidative modification of LDL that is responsible for the development and progression of atherosclerosis [12].

Several studies have shown that antioxidants are uniquely different from one another and work synergistically and more effectively [13–15]. Extensive literature survey has shown that there are no scientific reports regarding the effects of green tea and vitamin E in combination on serum and heart lipids, lipid metabolizing enzymes and histopathological alterations in myocardial infarction induced by isoproterenol. Hence, in this present study we evaluated the combined effect of green tea and vitamin E on serum and heart lipid profile along with histopathological alterations in isoproterenol-induced myocardial infarction in rats.

Material and Methods

Drugs and Chemicals

(\pm)-Isoproterenol hydrochloride was procured from Sigma Chemicals (St Louis, MO, USA).

Alcoholic extract of green tea (60% polyphenols) was obtained as gift sample from K. Patel Phyto Extractions Pvt. Ltd, Mumbai. Vitamin E (DL- α -Tocopherol acetate) and olive oil was purchased from Hi Media, India. All other chemicals used were of analytical grade.

Experimental Animals

Male Adult albino rats (Wistar strain) weighing between 200 and 230 g were used in the present study. All experiments were approved by the Institutional Animal Ethics Committee (IAEC) of the M. S. University of Baroda, India. The animals were housed in polyacredylin cages (38 × 23 × 10 cm) with not more than four rats per cage. They were housed in an air conditioned room and were kept in standard laboratory conditions under natural light and dark cycle (12h light and 12h dark) maintained at an ambient temperature 25±2°C. The animals were fed standard pellet diet (Amrut feeds, Pranav Agro Industries Ltd., Sangali, India) and water *ad libitum*. The number for approval of ethical committee is 404/01/a/CPCSEA and the proposal was approved by the meeting which was held on 15th February 2008.

Pilot study for dose fixation

Green tea at the doses of 25, 50 and 100mg/kg and vitamin E at the doses of 25, 50 and 100mg/kg were screened in isoproterenol induced myocardial infarction in rats. The optimum dose exhibiting maximum cardioprotective effect was evaluated by estimating serum lactate dehydrogenase, creatine phosphokinase-MB, serum Aspartate transaminase and serum Alanine transaminase levels and also the markers of oxidative stress such as Thiobarbituric acid reactive substances (TBARS) formation as a marker of lipid peroxidation, activities of reduced glutathione, superoxide dismutase and catalase. Green tea extract (100mg/kg) and vitamin E (100mg/kg) doses were found to be most effective in functional recovery of biochemical alterations. Hence these doses were selected for further evaluation alone as well as in combination in the isoproterenol induced myocardial infarction model (Data not shown).

Experimental Design

Animals were randomly allocated into six main groups comprising of eight animals in each group. Group I: Normal control rats received distilled water 2ml/kg for 30 days and normal saline subcutaneously on 29th and 30th day. Group II: Animals received olive oil as a vehicle for vitamin E (1ml/kg, p.o.) and normal saline on 29th and 30th day. Group III: Animals received ISO (200mg/kg, s.c.) on 29th and 30th day in normal saline. Group IV: Received green tea (100 mg/kg, p. o.) for 30 days and challenged with ISO on 29th and 30th day. Groups V: Received vitamin E (100 mg/kg, p. o.) in olive oil for 30 days and challenged with ISO on 29th and 30th day. Group VI: Received green tea (100 mg/kg, p.o.) and vitamin E (100 mg/kg, p.o.) in combination for 30 days and challenged with ISO on 29th and 30th day.

At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and lipid extraction was carried out for further estimations.

Extraction of Lipids from Heart Tissue

From the sample of heart tissue homogenate lipids were extracted by the method of Folch

et al (1957) [16]. To a known volume of tissue homogenate, 10ml of chloroform methanol mixture was added and mixed well for 30min using shaker and was filtered through Whatman filter paper (No.42) into a separating funnel. The filtrate was mixed with 0.2ml of physiological saline and the mixture was kept overnight undisturbed. The lower phase containing lipid was drained off into pre-weighted beaker. The upper phase was reextracted with more of chloroform-methanol mixture and the extract was pooled and evaporated under vacuum at room temperature. The lipid extract was re-dissolved in 3ml of chloroform methanol (2:1) mixture and the aliquots collected were used for the estimation of lipid levels.

Biochemical Estimation

Total cholesterol and triglyceride from heart lipid extracts were estimated using standard diagnostic kits (Reckon diagnostic Ltd, India). The content of free fatty acids and phospholipids from serum and heart lipid extract were estimated by the method of Horn and Menahan *et al* [17] and Fisker subbarow *et al* [18]. Blood was collected from the retro-orbital plexus under mild ether anesthesia. Serum was separated and total cholesterol (TC), triglyceride (TG), high Density lipoproteins (HDL-c) were determined by using standard diagnostic kits (Reckon Diagnostic Ltd., India). Low density lipoprotein (LDL-c) and very Low density lipoprotein cholesterol (VLDL-c) were determined by using Fridwald's Formula [19]. The activities of lipid metabolizing enzymes such as cholesterol ester synthetase (CES), lecithin: Cholesterol acyl transferase (LCAT) and lipoprotein lipase (LPL) were determined from the heart sample as suggested by Kothari *et al* [20], Hitz *et al* [21] and Slater *et al* [22].

Histological Examination

After decapitation, the heart was rapidly dissected out and washed immediately with saline and fixed in 10% buffered formalin. Hearts which were stored in 10% formalin were embedded in paraffin, sections cut at 5 μ m and were stained with haematoxyline and eosin. The sections of the heart were observed under microscope (Olympus BX10) for histological changes.

Statistical Analysis

Results are presented as mean \pm SEM. One-way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons using a computer based fitting program (Prism, Graph Pad). Differences were considered to be statistically significant when $P < 0.05$.

Results

The levels of total cholesterol, triglyceride, HDL-c, LDL-c, VLDL-c, free fatty acids (FFA) and phospholipids (PL) in control and experimental groups of rats are shown in Table 1. Rats intoxicated with ISO showed a significant ($p < 0.01$, $p < 0.001$) increase in the levels of TC, TG, FFA and PL with the levels of HDL-c being an exception where there was a significant ($p < 0.01$) decrease. Treatment with green tea and vitamin E in combination for 30 days significantly ($p < 0.01$, $p < 0.001$) decreased the levels of total cholesterol, triglycerides, LDL-c, VLDL, free fatty acids and phospholipids with subsequent increase in the level of HDL-c cholesterol as compared to the ISO treated group.

Tab. 1. Effect of green tea and vitamin E in combination on serum lipid profile in ISO induced myocardial infarction

Groups	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	FFA (mg/dl)	PL (mg/dl)
I	92.50 ± 4.23	50.56 ± 2.21	38.58 ± 2.99	43.81 ± 4.86	10.11 ± 0.44	36.31 ± 3.17	111.4 ± 3.48
II	86.06 ± 5.95	48.88 ± 1.33	36.96 ± 2.95	44.34 ± 3.86	9.77 ± 0.26	35.57 ± 4.62	100.1 ± 5.37
III	139.8 ± 5.64 ^{***}	78.79 ± 5.61 ^{***}	22.10 ± 1.92 ^{**}	102.0 ± 5.16 ^{***}	15.75 ± 1.12 ^{***}	64.00 ± 4.89 ^{**}	157.5 ± 4.03 ^{***}
IV	113.2 ± 5.03 ^{^^}	61.07 ± 3.16 ^{^^}	34.85 ± 1.32 [^]	67.27 ± 4.69 ^{^^^}	12.21 ± 0.62 ^{^^}	38.79 ± 2.95 ^{^^}	135.6 ± 3.61 ^{^^}
V	116.7 ± 5.49 [^]	63.45 ± 2.63 [^]	35.06 ± 4.27 [^]	68.97 ± 3.02 ^{^^^}	12.79 ± 0.58 [^]	41.49 ± 4.30 [^]	133.3 ± 4.14 ^{^^}
VI	94.73 ± 3.46 ^{^^^}	53.35 ± 1.94 ^{^^^}	36.87 ± 2.54 ^{^^}	47.09 ± 3.44 ^{^^^}	10.76 ± 0.41 ^{^^^}	38.84 ± 3.87 ^{^^}	115.9 ± 3.21 ^{^^^}

Values are expressed as Mean ± SEM, (n=6), *P<0.05, **P<0.01, ***P<0.001 values compared to control groups, ^P<0.05, ^^P<0.01, ^^P<0.001 values compared to ISO groups, P>0.05 considered as non significant (ns). Group I: normal control, Group II: olive oil, Group III: ISO; Group IV: GT+ ISO, Group V: vitamine E+ISO, Group VI: GT+vitamine E+ISO.

Table 2 shows the levels of total cholesterol, triglyceride, free fatty acids and phospholipids of hearts in normal and experimental groups of rats. Rats treated with ISO showed a significant ($p<0.01$, $p<0.001$) increase in the levels of TC, TG, FFA with a significant decrease ($p<0.05$) in the levels of phospholipids as compared to normal control animals. Treatment with green tea and vitamin E in combination for 30 days resulted in a significant ($p<0.01$, $p<0.05$) decrease in the levels of total cholesterol, triglycerides, free fatty acids and subsequent increase in phospholipids level as compared to ISO treated group.

Tab. 2. Effect of green tea and vitamin E in combination on Heart lipid profile in normal and ISO induced myocardial infarcted rats

Groups	TC (mg/g wet tissue)	TG (mg/g wet tissue)	PL (mg/g wet tissue)	FFA (mg/g wet tissue)
Group I	8.93± 0.94	6.33± 0.72	25.39± 1.75	0.94± 0.07
Group II	8.23± 0.63	5.94± 0.63	25.62± 1.98	0.87± 0.08
Group III	13.69± 1.34 ^{**}	11.26± 0.51 ^{***}	16.37± 1.35 [*]	1.45± 0.11 ^{**}
Group IV	10.01± 0.62 [^]	7.82± 0.62 [^]	24.60± 1.85 [^]	1.03± 0.05 [^]
Group V	10.08± 0.43 [^]	7.33± 0.82 ^{^^}	24.64± 1.75 [^]	1.10± 0.08 ^{ns}
Group VI	9.14 ± 0.67 ^{^^}	7.50± 0.49 ^{^^}	25.70± 2.48 [^]	0.97± 0.09 [^]

Values are expressed as Mean ± SEM, (n=6), *P<0.05, **P<0.01, ***P<0.001 values compared to control groups, ^P<0.05, ^^P<0.01, ^^P<0.001 values compared to ISO groups, P>0.05 considered as non significant (ns). Group I: normal control, Group II: olive oil, Group III: ISO; Group IV: GT+ ISO, Group V: vitamine E+ISO, Group VI: GT+vitamine E+ISO.

Fig 1, 2 and 3 show the change in the activities of lipid metabolizing enzymes such as cholesterol ester synthetase, lecithin cholesterol acetyl transferase and lipoprotein lipase. ISO intoxication showed a significant ($p < 0.001$) increase in the levels of CES and a significant ($p < 0.001$, $p < 0.01$) decrease in the level of LCAT and LPL as compared to control animals. Treatment with green tea and vitamin E in combination for 30 days significantly ($p < 0.01$) increased the levels of LPL and LCAT whereas the level of CES is also significantly ($P < 0.05$) decreased as compared to the ISO group.

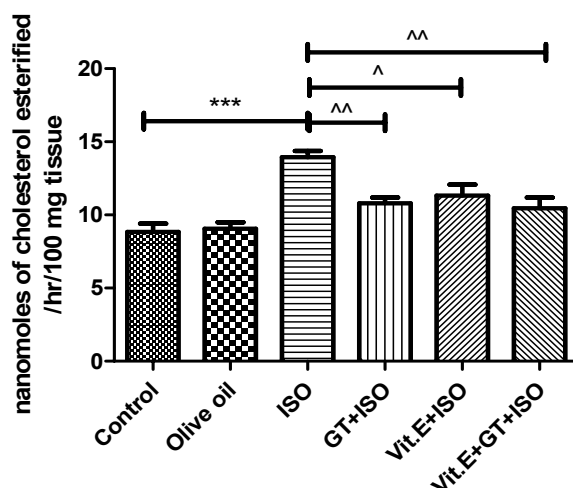


Fig. 1. Effect of green tea and vitamin E combination on the activity of cholesterol ester synthetase in ISO induced myocardial infarction

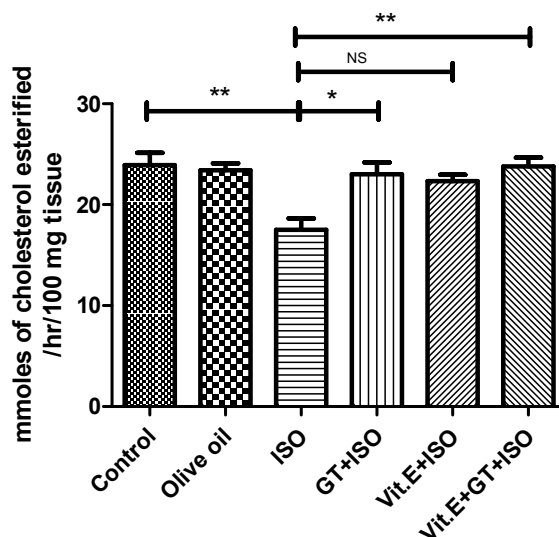


Fig. 2. Effect of green tea and vitamin E combination on the activity of LCAT in ISO induced myocardial infarction.

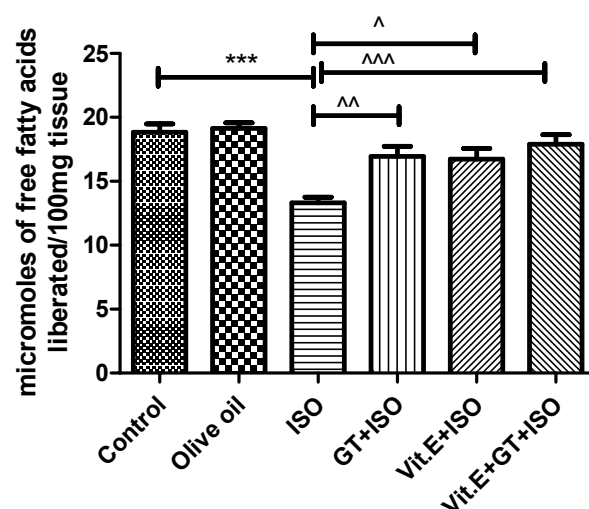


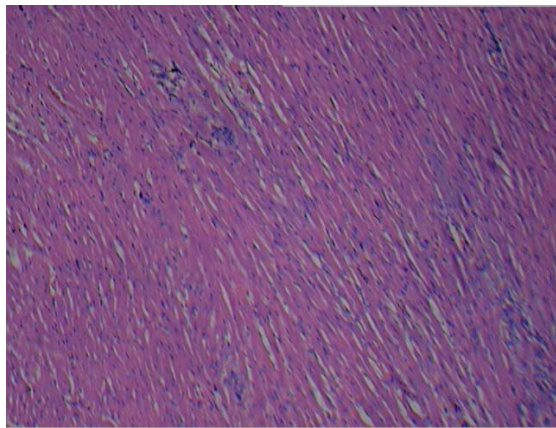
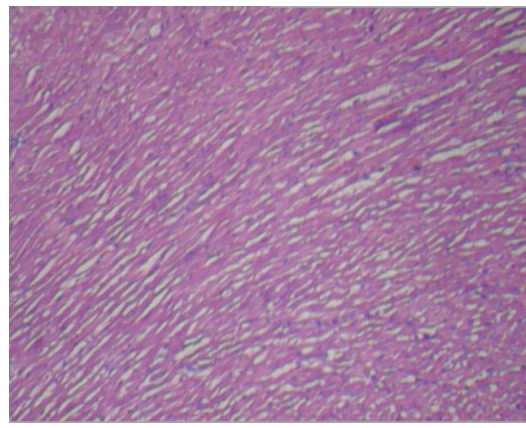
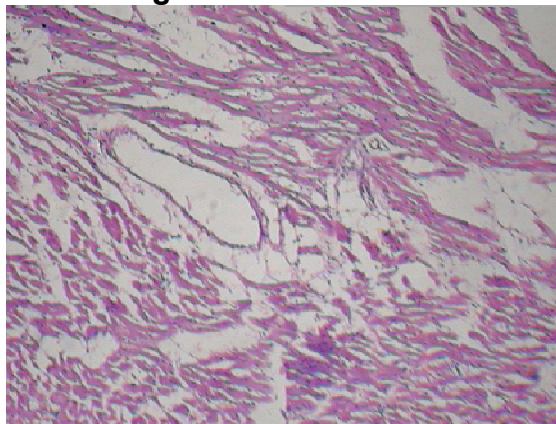
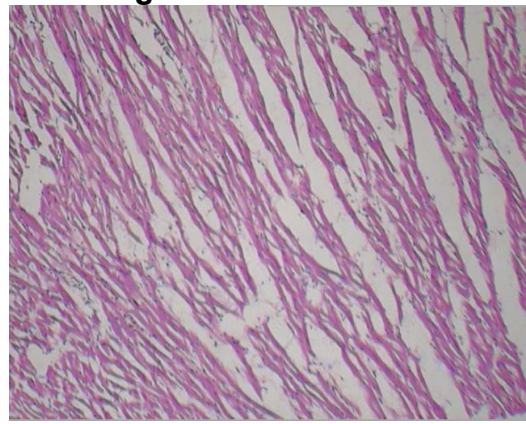
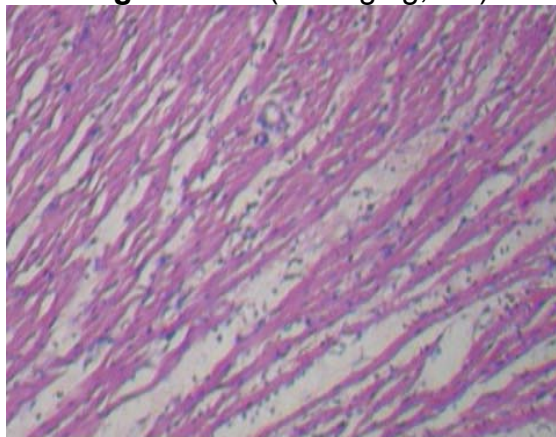
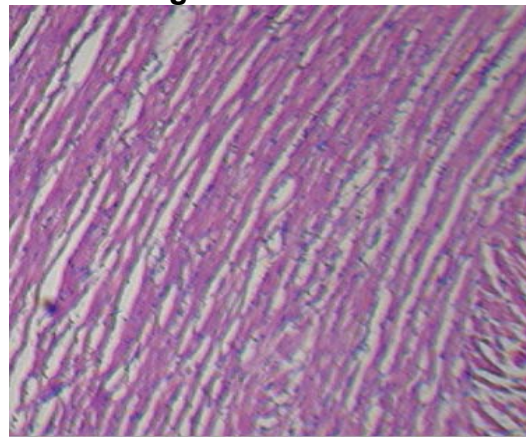
Fig. 3. Effect of green tea and vitamin E combination on the activity of lipoprotein Lipase in ISO induced myocardial infarction

Fig. 1, 2 and 3. Values are expressed as Mean \pm SEM, (n=6), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ values compared to control groups, ^ $P < 0.05$, ^^ $P < 0.01$, ^^ $P < 0.001$ values compared to ISO groups, $P > 0.05$ considered as non significant (ns).

Fig. 4 shows histopathological alteration in ISO intoxicated rats. ISO administration resulted in severe necrotic changes along with oedema, inflammatory cells and marked fragmentation of muscle fibers (Fig. 4a) in rat hearts as compared to control animals (Fig. 4c). Green tea and vitamin E combination treated rats exhibited a decreased degree of necrosis with less fragmentation of fibers (Fig. 4f) as compared to green tea and vitamin E treated animals, which shows the cardioprotective effects of the combination against ISO induced cardiac necrosis.

Discussion

Lipids play an important role in cardiovascular disease, by modifying the composition, structure and stability of cell membranes. Altered lipid metabolism is considered to accelerate the development of atherosclerosis, a major risk factor in myocardial infarction. High levels of circulating cholesterol and its accumulation in heart tissue is well associated with cardiovascular damage [22]. The main target available for reactive oxygen species for attack is polyunsaturated fatty acids (PUFA), which is the precursor for lipid peroxide formation. Lipid peroxide elevation could be attributed to the accumulation of lipids in the heart [23]. In our previous study, we observed a significant increase in the level of lipid peroxidation in ISO intoxicated rats. Combination of vitamin E and green tea significantly decreased the increased level of lipid peroxidation [24]. In the present study, rats intoxicated with ISO showed increased in the levels of serum and heart tissue lipids and decreased heart tissue phospholipids and serum HDL cholesterol levels. The observed changes in cholesterol, triglycerides, phospholipids and free fatty acids in the serum and heart agree with previous reports [25]. ISO induced elevation in cholesterol levels could be due to increase in biosynthesis and decrease in its utilization. ISO induces free radical formation which may cause cellular cholesterol accumulation by increasing cholesterol biosynthesis, by decreasing cholesteryl ester hydrolysis and by reducing cholesterol efflux [26]. Combined treatment with green tea and vitamin E showed a better protection than the antioxidative components alone.

**Fig. 4a.** Normal control**Fig. 4b.** Vehicle control**Fig. 4c.** ISO (200mg/kg, s.c)**Fig. 4d.** Vit.E+ISO**Fig. 4e:** GT+ISO**Fig. 4f:** Vit.E+GT+ISO

- Fig. 4.** A Photomicrograph of a section in cardiac muscle (H&E staining, magnification X10): (a and b) represents myocardium of control and vehicle treated rats showing normal architecture. (c) Isoproterenol alone treated myocardium showing zone with oedema and inflammatory cells and separation of muscle fibres. (d and e) Vitamin E +ISO and GT+ISO treated myocardium showing mild oedema with less fragmentation of muscle fibres and inflammatory cells.(f) Vit.E + green tea+ISO showing comparatively less oedema, inflammatory cells and separation of muscle fibres to that of d and e.

In the present study there was a significant decrease in cardiac LCAT and LPL activity whereas a significant increase in the activity of CES was found in ISO intoxicated rats. HDL is the main substrate for LCAT for cholesterol esterification and incorporation [27]. A significant increase in the level of HDL in rats treated with green tea and vitamin E alone and in combination support the increase in cardiac LCAT activity. LPL in the heart is involved in the uptake of TG rich Lipoproteins from circulation [28]. An inverse correlation between TG and LPL activity has been reported [29]. In the present study hypertriglyceridemia observed in ISO intoxicated rats is due to decrease activity of LPL in the myocardium resulting in decreased uptake of TG from the circulation. Accumulation of ester cholesterol occurs when the rate of esterification by cholesterol ester synthetase exceeds the rate of hydrolysis, which in turn results in myocardial membrane damage [30]. Green tea and vitamin E in combination alters the activities of LCAT, LPL and CES near to the normal by increasing HDL and decreasing TG and cholesterol levels, indicating the potential lipid lowering effects of green tea and vitamin E combination. Further the combination of green tea and vitamin E also protected the histological changes which occur during ISO intoxication. Our earlier study [24] has also reported that green tea and vitamin E used in combination protects changes in biomarkers of oxidative stress and membrane bound ATPases synergistically in ISO induced myocardial infarction.

Both green tea and vitamin E are dietary compounds, and their antioxidant properties are thought to be the common reason that they are pharmacologically useful against heart diseases. Green tea is particularly rich in epigallocatechin, a powerful antioxidant, which exerts a protective effect against LDL oxidation [31]. It is known that the oxidatively modified LDL has a central role in atherosclerosis. The significant hypocholesterolemic effect of green tea is attributed to a reduction in cholesterol absorption and to an increased excretion of biliary acids and cholesterol and also the inhibition of cholesterol synthesis in liver [32]. Vitamin E is a lipid soluble chain breaking antioxidant in human plasma and low density lipoprotein [12]. Vitamin E has been reported to produce a stabilizing effect on heart phospholipids by preventing changes in fatty acid composition and peroxidative deterioration. Green tea is a water soluble antioxidant and contains polyphenols, which are reported to donate hydrogen atoms to tocopheryl radicals, and enhances the antioxidant efficiency of alpha tocopherol and thereby regenerates tocopherol [33]. This may possibly be the reason for the protective effect of green tea and vitamin E combination in attenuating cardiac dysfunction through maintaining lipid profile and histological alteration in ISO intoxicated rats.

Finally concluding, the result of the present study indicates that the combined treatment with green tea and vitamin E prevents the ISO-induced myocardial infarction better than green tea and vitamin E alone. For prevention of myocardial infarction, herbal drugs containing polyphenols and vitamin E in combinations might be effective.

Index of abbreviations

TC: Total cholesterol

TG: Triglyceride

HDL-c: High density lipoprotein cholesterol

LDL-c: low density lipoprotein cholesterol

VLDL-c: very low density lipoprotein cholesterol

LPL: lipoprotein lipase

CES: cholesterol ester synthetase

LCAT: lecithin: cholesterol acyl transferase

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Authors' Statements

Competing Interests

The authors declare no conflict of interest.

Animal Rights

The institutional and (inter)national guide for the care and use of laboratory animals was followed. See the experimental part for details.

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