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EVALUATION AND INTERACTION OF GREEN TEA EXTRACT ON MYOCARDIAL POTENCY OF BETA BLOCKER USING ISOPROTERENOL INDUCED MYOCARDIAL NECROSIS MODEL

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ABSTRACT

As now a days we are consuming both herbals and modern medicines at same time so herb-drug interaction arises. Carvedilol, which we use in various cardiovascular disorders itself has various side effect. Green tea has high polyphenol content and is used in various cardiovascular disorders. Hence the present study was conducted to find out the beneficial effect of green tea extract and its interaction with carvedilol in isoproterenol induced myocardial necrosis model in rats. At the end of the study, green tea extract brought a significant improvement in SOD, CATALASE, TBARS, LDH, CKMB, CKNAC, ECG parameters. When it was administered simultaneously with carvedilol, it showed a synergistic effect. Hence the dose of carvedilol can be reduced if one consumes specified amount of green tea along with it. Reduced dose of carvedilol means less side effects to the body arising from it.

INTRODUCTION

Towards the end of this century, the use of herbals in medicine is seen. In traditional medicines these herbs were used safely. But safety interaction arises, when we mix these with our modern times medicines[1]. In developing countries, persons suffering and dying from cardiac diseases is on higher side. India also plays an important role in it [2].

Green tea is made from the unfermented leaves of the *Camellia sinensis* plant. In green tea highest portion of active polyphenols are present. This is in comparison to oolong and black tea. Carvedilol is a non selective beta and alpha 1 blocker. It is indicated in the management of several cardiovascular disorders such as congestive heart failure, myocardial infraction, hypertension. But this drug has itself various side effects including the cardiovascular ones which is reported in various worldwide clinical studies. The non cvs side effects are dizziness, fatigue, diarrhea, nausea, vomiting, increased cough, allergy etc. The side effects related to cardiovascular are worsening of congestive heart failure, hypertension, myocardial infraction-angina pectoris, arrhythmias[3, 4, 5,6, 7, 8, 9, 10, 11, 12, 13]. Tea polyphenols help in preventing various cardiovascular diseases by a variety of mechanisms[14, 15, 16, 17].

Hence, present study is designed to demonstrate different pharmacological interactions between green tea extract and carvedilol.

MATERIALS AND METHOD

Animals

The animals used in this experiment was sanctioned by Institutional Animal Ethical Committee which approved the protocol also. Healthy male wistar rats were used. They were kept hygienically in polypropylene cages in college animal house. Temperature of 25±3˚C, humidity of 55±5%, 12hr day/night cycle and acclimatization period of 1 week were maintained. Standard pellet feed for rats was provided with water ad libitum. Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines were followed.

Extract preparation and chemicals

Green tea extract was prepared from green tea leaves (*Camellia sinensis*) which was obtained from the local market of Mangalore. Here aqueous extract was taken into

consideration. Our interest product was thick gummy mass. For dosing to animals it was dissolved in distilled water[18]. Isoproterenol was procured from Sigma Aldrich, India. Carvedilol used as marketed tabletsCardivas from Sun Pharma, India. All other chemicals and kits used were of standard laboratory and professional analytical grade.

Dose selection

According to OPPTS (Office of Prevention, Pesticide and Toxic Substance) guidelines – limit test, for acute toxicity studies, the dose selection study was carried out. Overnight fasted mice were taken. 1/10th and 1/50th of the maximum safe dose were selected as high and low doses respectively for the present study. According it was found out to be 500 and 100 mg/kg body weight[19].

Groupings

The rats were divided into following groups of eight animals each as following:

Group No.	Description
I	animals kept as normal control without pretreatment
II	ISO (isoproterenol) only (toxic control)
III	animals treated with Carvedilol (CDL) for 1 week and subjected to ISO toxicity
IV	animals treated with low dose of GTE (green tea extract) for 4 weeks + ISO
V	animals treated with high dose of GTE for 4 weeks + ISO
VI	CDL will be added during last week of treatment with low dose of GTE (4 weeks) + ISO.
VII	CDL will be added during last week of treatment with high dose of GTE (4 weeks) + ISO.

Isoproterenol induced myocardial necrosis in rats [20]

After the end of treatment period, isoproterenol (150 mg/kg s.c) was administered for two consecutive days. 48 hours after the 1st dose of isoproterenol, blood was taken out by retro orbital route under anesthesia. Measurement of LDH, CKMB (creatinine kinase) was done. Heart was isolated 2hrs after the last dose of drug to prepare heart tissue homogenate under anesthesia. Measurement of SOD (superoxide dismutase), CAT

(catalase), TBARS (thiobarbituric acid reactive substances) in heart tissue homogenate, LDH, CKMB, CKNAC in serum were done. Carvedilol dose was 10mg/kg body weight.

ECG studies [21]

After anesthetizing the rat with a combination of ketamine hydrochloride (75mg/kg, i.p) and xylazine (8.0mg/kg, i.p), leads were attached to the dermal layer of both the front paws and hind legs. Recording was made with the help of computerized ambulatory ECG system-modified physiography (INCO). Parameters such as heart rate, QRS duration, QT segment, RR interval, PR interval were measured. Animal grouping and treatment was same as ISO model.

RESULTS

Effect on ECG parameter in ISO model

Treatment with ISO brought extremely significant ($P<0.001$) increase in heart rate, PR, QRS, RR, QT intervals when compared with NORMAL. All the treatment groups showed extremely significant ($P<0.001$) improvement in respective parameters when compared with ISO group. In the above ECG parameters significant levels varied from significant ($P<0.05$) to moderately significant ($P<0.01$) to extremely significant ($P<0.001$) in these groups (GTE100+CDL+ISO and GTE500+CDL+ISO) when compared with CDL. See tables 1, 2.

Effect on SOD, CAT, TBARS in heart tissue homogenate against ISO model

Treatment with ISO brought extremely significant ($P<0.001$) increase in TBARS parameter and moderately significant ($P<0.01$) decrease in CAT parameter and extremely significant ($P<0.001$) decrease in SOD parameter when compared with NORMAL. Treatment groups showed moderately significant ($P<0.01$) to extremely significant ($P<0.001$) improvement in the SOD parameter when compared with ISO group. The CAT and TBARS levels significantly varied from significant ($P<0.05$) to moderately significant ($P<0.01$) to extremely significant ($P<0.001$) in these groups (GTE100+CDL+ISO and GTE500+CDL+ISO) when compared with ISO. The TBARS level showed extremely significant ($P<0.001$) improvement in the parameter in these groups (GTE100+CDL+ISO and GTE500+CDL+ISO) when compared with CDL. See table 3.

Effect on LDH, CKMB, CKNAC level in serum against ISO model.

Treatment with ISO brought extremely significant (P<0.001) increase in LDH, CKMB, CKNAC levels in serum when compared with NORMAL. All the treatment groups showed extremely significant (P<0.001) improvement in the above parameters when compared with ISO group. In the above cardiac biomarkers significant levels varied from significant (P<0.05) to moderately significant (P<0.01) to extremely significant (P<0.001) in these groups (GTE100+CDL+ISO and GTE500+CDL+ISO) when compared with CDL. See table 4.

TABLE 1- EFFECT ON ECG PARAMETER IN ISO MODEL

GROUP	HEART RATE	PR INTERVAL (MS)	QRS INTERVAL (MS)	RR INTERVAL (MS)	QT INTERVAL (MS)
NORMAL	150±2.1	130±4.6	125±2.1	200±3.7	200±4.6
ISO	425±4.6***	350±5.2***	250±3.4***	330±4.4***	400±2.9***
CDL+ISO	220±8.9***###	230±3.9***###	170±5.6***###	231±2.6*###	250±3.1***###
GTE100+ISO	254±5.2***###	290±6.7***###	190±8.2***###	255±8.1***###	270±5.2***###
GTE500+ISO	237±6.4***###	265±8.4***###	183±7.5***###	243±7.9***###	265±7.8***###
GTE100+CDL+ISO	169±7.3###+++	140±7.3###+++	144±4.3###+	219±5.2###	220±6.4###++
GTE500+CDL+ISO	160±4.8###+++	125±5.8###+++	130±3.7###+++	210±6.3###	210±5.6###+++

VALUES ARE EXPRESSED AS MEAN ±SEM. N=6. ***P<0.001 WHEN COMPARED NORMAL. ###P<0.001 WHEN COMPARED WITH ISO. +P<0.05, ++P<0.01, +++P<0.001 WHEN COMPARED WITH CDL.

TABLE 2- EFFECT ON ECG PARAMETER IN ISO MODEL

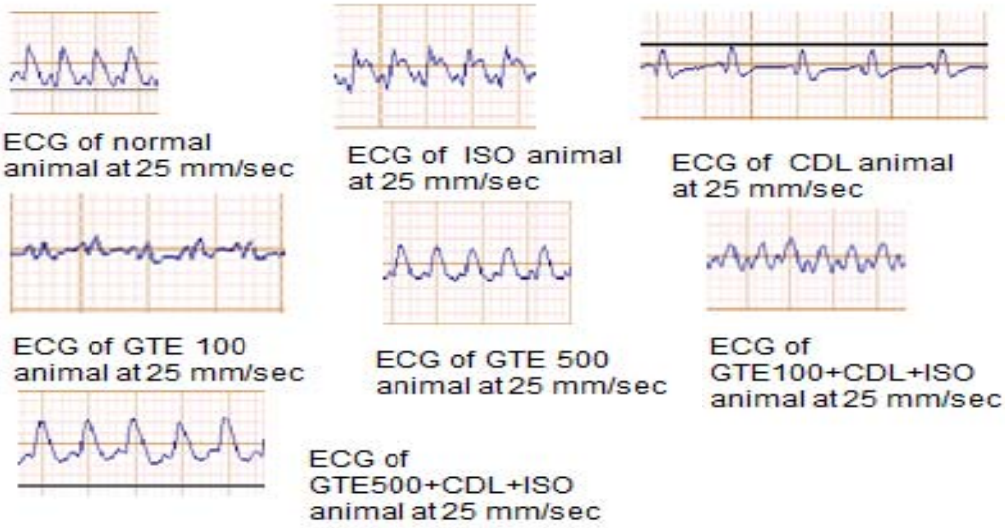

TABLE 3- EFFECT ON SOD, CATALASE, TBARS IN HEART TISSUE HOMOGENATE AGAINST ISO MODEL

GROUP	SOD (U/MG)	CAT (U/MG)	TBARS (U/MG)
NORMAL	7.29±0.32	5.1±0.9	1.58±0.48
ISO	0.21±0.45***	1.1±0.6**	7.98±0.17***
CDL+ISO	6.49±0.93###	4.1±0.5	5.96±0.63***#
GTE100+ISO	4.89±0.71###	3.2±0.7	6.71±0.59***
GTE500+ISO	5.98±0.56###	3.7±0.5	6.43±0.36***
GTE100+CDL+ISO	6.98±0.14###	4.5±0.8#	2.32±0.31###+++
GTE500+CDL+ISO	7.15±0.27##	4.9±0.7##	1.87±0.27###+++

VALUES ARE EXPRESSED AS MEAN ±SEM. N=6. **P<0.01, ***P<0.001 WHEN COMPARED WITH NORMAL. #P<0.05, ##P<0.01, ###P<0.001 WHEN COMPARED WITH ISO. +++P<0.001 WHEN COMPARED WITH CDL.

TABLE 4- EFFECT ON LDH, CKMB, CKNAC LEVEL IN SERUM AGAINST ISO MODEL

GROUP	CKNAC (U/LIT)	CKMB (U/LIT)	LDH (U/LIT)
NORMAL	188.23±1.65	115.27±7.43	963.47±23.32
ISO	1000.00±5.33***	2502.11±2.59***	5010.02±36.79***
CDL+ISO	305.83±3.48***###	288.83±3.84***###	1420.38±41.86***###
GTE100+ISO	439.17±2.84***###	389.18±6.57***###	1709.51±53.45***###
GTE500+ISO	371.54±7.36***###	334.47±9.71***###	1501.68±48.57***###
GTE100+CDL+ISO	250.67±4.51***###+++	249.35±5.22***###++	1358.57±29.32***###
GTE500+CDL+ISO	210.46±3.71*###+++	191.86±8.63***###+++	1206.43±55.84**###+

VALUES ARE EXPRESSED AS MEAN ±SEM. N=6. *P<0.05, **P<0.01, ***P<0.001 WHEN COMPARED WITH NORMAL. ###P<0.001 WHEN COMPARED WITH ISO. +P<0.05, ++P<0.01, +++P<0.001 WHEN COMPARED WITH CDL.

DISCUSSION[20, 22]

Isoproterenol is a beta adrenergic agonist. It is also a synthetic catecholamine. This compound induce severe stress and produce myocardial injury in the cardiac muscle fibre. This then leads to a disease which resemble as myocardial infraction. Isoproterenol produce myocardial infraction in a variety of ways. There will be formation of reactive free radicals due to oxidative metabolism of catecholamines. This will cause increase in oxidative stress. Also it will cause lipid peroxidation. This will cause damage to the cell membrane. Once the cell membrane gets damaged their will be alterations in the level of

enzymes in the body. Their will be decrease in antioxidant levels in the body which will further damage the myocardium.. Superoxide dismutase, catalase, thiobarbituric acid reactive substances which should remain inside the heart tissue will come out. Creatinine kinase and LDH levels will rise in serum. Free radicals such as reactive oxygen species can induce necrosis or apoptosis. Chances are there that it may kill the cells and further worsen the myocardial injury. Also it can produce inflammatory reaction involving mast cells. Due to ischemia-necrosis there will be formation of cardiac lesions and change in ECG parameters. Green tea cause improvement in all these parameters. High dose is more effective than low dose. Also it shows synergistic action with carvedilol. If one is consuming green tea then the dose of carvedilol can be reduced. This will lessen the toxic side effects of the drug in the body.

CONCLUSION

Green tea extract did not show any signs of toxicity in toxicity study. It improved the conditions of the animals suffering from myocardial infraction and its cascade effects. It showed synergistic action with carvedilol.

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