International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 4(3): May-June 2014

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 28-05-2014; Revised; Accepted: 30-06-2014

EVALUATION AND INTERACTION OF GREEN TEA EXTRACT ON MYOCARDIAL POTENCY OF BETA BLOCKER USING ISCHEMIA REPERFUSION INDUCED MYOCARDIAL DAMAGE MODEL

Gupta K[1]*, Kamath J V[2]

1. Research Scholar, Bhagwant University, Ajmer, Rajasthan.
2. Shree Devi College of Pharmacy, Mangalore, Karnataka.

Keywords:

Green tea, Carvedilol, Ischemia

For Correspondence:

Gupta K

Research Scholar,

Bhagwant University,

Ajmer, Rajasthan

E-mail:

pharmacology1985@yahoo.com

ABSTRACT

As now a days we are consuming both herbals and modern medicines at same time so herb-drug interaction arises. There are chances of herb drug interaction because we are taking both herbals and modern day medicines simultaneously. Carvedilol is a drug used in many cardiovascular disorders. But this drug has numerous side effects. 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. In many cardiovascular disorders green tea is used. it contains high polyphenols. So the present study was conducted to find out the beneficial effect of green tea extract and its interaction with carvedilol in ischemia reperfusion induced myocardial damage model in rats. At the end of the study, green tea extract brought a significant improvement in SOD, CAT, TBARS, LDH, CKMB, CKNAC, % RECOVERY parameters. When it was administered along with carvedilol, it showed a synergistic effect. So carvedilol dose can be reduced if one consumes specific quantity of green tea along with it. Reduced dose of carvedilol means less toxic effects to the body arising from it.

INTRODUCTION

Because the herbs have a strong age old traditional background, practioners prescribe it. They keep in mind its pharmacological therapeutic value and its safety. Hence there is increase in its popularity. But this boon is also causing some effects such as drug interaction. This interaction can be good or bad [1,2].

From the unfermented leaves of the *Camellia sinensis* plant, green tea is obtained. It contains highest portion of active polyphenols in comparison to oolong and black tea. Carvedilol is a non selective beta and alpha 1 blocker. It is used in pharmacotherapy of many cardiovascular disorders such as congestive heart failure, myocardial infraction, hypertension. Carvedilol has got non cardiovascular (dizziness, fatigue, diarrhea, nausea, vomiting, increased cough, allergy etc.) and cardiovascular (worsening of congestive heart failure, hypertension, myocardial infraction-angina pectoris, arrhythmias) side effects [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. The polyphenols present in tea prevent many cardiovascular diseases by numerous mechanisms[14, 15, 16, 17].

So, the present study is aimed to find out various pharmacological interactions between green tea extract and carvedilol.

MATERIALS AND METHOD

Animals

Institutional Animal Ethical Committee passed the protocol and animal required. Research work was carried out at Shree Devi College of Pharmacy, Mangalore. Healthy male wistar rats were kept hygienically in polypropylene cages in college central animal house. 25±3˚C temperature, 55±5% humidity, 12hr day/night cycle, acclimatization period of 1 week were followed. Rats were fed with standard rat pellet feed and water ad libitum. Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines were taken into consideration.

Extract preparation and chemicals

Green tea leaves (*Camellia sinensis*) were purchased from Mangalore market. Its aqueous extract was made. It was then concentrated to thick gummy mass. For animal dosing it was dissolved in distilled water[18]. Marketed tablets of carvedilol, Cardivas from Sun Pharma, India was used. Standard laboratory and analytical grade chemicals and kits were used.

Dose selection

Dose selection study was carried out as per OPPTS (Office of Prevention, Pesticide and Toxic Substance) guidelines – limit test, for acute toxicity studies on overnight fasted mice.

High and low doses selected in this study were 1/10th and 1/50th of the maximum safe dose respectively which were 5oo and 100 mg/kg body weight[19].

Groupings

This is in general the groupings. Number of rats in each group were eight:

NUMBER	NAME
1	NORMAL CONTROL
2	TOXIC CONTROL (ISO)
3	CDL P.O FOR 1 WEEK + ISO
4	GTE (LD) FOR 4 WEEKS + 1SO
5	GTE (HD) FOR 4 WEEKS + ISO
6	GTE (LD) FOR 4 WEEKS + CDL (LAST 1 WEEK) + ISO
7	GTE (HD) FOR 4 WEEKS + CDL (LAST 1 WEEK) + ISO

ISCHEMIA REPERFUSION (IRI) INDUCED MYOCARDIAL DAMAGE IN RATS

A modified langendorff's setup for the isolated perfused heart was made and used for study. The system consists of water jacketed coil with a three-way connecter inlet and three-way connecter outlet. The lower limb of the outlet passes through the stopper for cannulation of the aorta. A latex injecting port is provided between the stopper and outlet. The upper limb of the outlet traps the air bubble. A monobloc water pump circulates the water at 37˚c from a water bath to the water-jacketed apparatus and back to the water bath. Krebs-Henseleit (K-H) passes through the lower limb of inlet, spiral tube and lower limb of the outlet before entering the aorta. The upper limb of the inlet is also traps the air bubbles also. Animals were anesthetized with ketamine (70 mg/kg, *i.p*) and xylazine (10 mg/kg, *i.p*). Heart was quickly removed and mounted on a modified Langendorff's apparatus through the aorta. Animals were heparinized with heparine (100 IU) before 30 min of anesthesia.

Retrograde perfusion was started with modified Krebs Heinseleit (KH) buffer solution (pH 7.4) bubbled with carbogen (95% O2 + 5% CO2); at a flow rate of 5.5 ml/min using a regulated perfusion pump.

The modified K-H solution was filtered through wattman filter paper 1 before use to avoid micro particles, which obstruct the coronary arteries. The pH of K-H solution was adjusted to 7.4 to avoid K-H buffer acidosis that may occur after prolonged gassing with carbogen. The heart was mounted and perfused through the aorta with flow rate of 5.5 ml/min at 37˚c by means of a roller perfusion pump. A fine thread was tied to the apex of the heart and passed through a thermostatically controlled water-jacketed moist chamber and through a pulley to the force transducer. The chamber was easy to position at the bottom of the system one the isolated heart was cannulated. In the thermostatic chamber, the heart was allowed to equilibrate for 10 minutes and then regular recording were taken for perfusion period of 15 minutes. Measurement of contractile force was done with force displacement transducer and recorded in the computer. After the initial pre ischemic period of 20 minutes the heart was subjected to K-H solution. The recording of contractile force and heart rate was done. After pre ischemic period the heart was subjected 15 min of global no-flow ischemia by blocking the flow of modified K-H solution and carbogen supply followed by the post-ischemic developed tension was recorded for 15minutes. Perfusate were collected at post ischemic and were used for the determination of diagnostic marker enzymes. The heart were removed for investigation. Susceptibility or vulnerability of the ischemic heart to reperfusion injury was estimated by comparing the pre and post reperfusion effect amongst the treatment group with respect to control. Responses were recorded using a force transducer connected to a computer with a resting tension of 2 gm where the developed tension was equivalent to 2 gm. The recording speed was adjusted to minimum 0.05mm/sec and the sensitivity of transducer was adjusted to maximum i.e. 0.1 mv. [20,21,22] Superoxide dismutase[23], catalase[24], thiobarbituric acid reactive substances[25] were measured. With the help of kits lactate dehydrogenase, creatinine kinases (CKMB, CKNAC) were measured.

RESULTS

Effect on SOD, CATALASE, TBARS in heart tissue homogenate against IRI induced myocardial infraction

Treatment groups showed extremely significant ($P<0.001$) improvement in the SOD parameter when compared with IRI group. The CAT level showed significant result ($P<0.05$) in GTE 500 group and extremely significant result ($P<0.001$) in CDL, GTE100+CDL, GTE500+CDL groups when compared with IRI group. Treatment groups showed extremely significant ($P<0.001$) improvement in the TBARS parameter when compared with IRI group. See table 1.

Effect on LDH, CKMB, CKNAC level in perfusate against IRI induced myocardial infraction

Treatment groups showed extremely significant (P<0.001) improvement in CKNAC, CKMB, LDH parameters when compared with IRI group. GTE100+CDL group showed extremely significant (P<0.001) improvement in CKNAC, CKMB and significant result (P<0.05) in LDH parameter when compared with CDL group. GTE500+CDL group showed extremely significant (P<0.001) improvement in CKNAC, CKMB, LDH parameters when compared with CDL group. See table 2.

Effects on percentage recovery in terms of heart rate and developed tension against ischemia reperfusion induced myocardial damage

Treatment groups showed extremely significant (P<0.001) improvement in % recovery in terms of heart rate and developed tension parameters when compared with IRI group. GTE100+CDL, GTE500+CDL groups showed extremely significant (P<0.001) improvement in % recovery in terms of heart rate parameter when compared with CDL group. GTE100+CDL, GTE500+CDL groups showed moderately significant (P<0.01) improvement in % recovery in terms of developed tension parameter when compared with CDL group. See table 3.

TABLE 1. EFFECT ON SOD, CATALASE, TBARS IN HEART TISSUE HOMOGENATE AGAINST IRI INDUCED MYOCARDIAL INFRACTION

GROUP	SOD (U/MG)	CAT (U/MG)	TBARS (U/MG)
IRI	1.00±0.02	1.1±0.4	15.71±0.17
CDL	7.53±0.24###	4.7±0.5###	2.3±0.58###
GTE100	6.67±0.41###	2.9±0.8	3.0±0.81###
GTE500	7.10±0.37###	3.6±0.7#	1.6±0.32###
GTE100+CDL	8.04±0.31###	5.5±0.1###	1.71±0.46###
GTE500+CDL	8.47±0.03###	6.2±0.3###	1.16±0.57###

VALUES ARE EXPRESSED AS MEAN±SEM. N=6. #P<0.05, ###P<0.001 WHEN COMPARED WITH IRI.

TABLE 2. EFFECT ON LDH, CKMB, CKNAC LEVEL IN PERFUSATE AGAINST IRI INDUCED MYOCARDIAL INFRACTION

GROUP	CKNAC (U/LIT)	CKMB (U/LIT)	LDH (U/LIT)
IRI	836.43±4.52	800.41±8.33	1000.44±18.36
CDL	260.97±8.73###	240.86±2.44###	290.86±11.88###
GTE100	307.45±3.29###	275.84±8.93###	370.71±12.35###
GTE500	280.83±2.17###	299.11±6.43###	330.22±13.48###
GTE100+CDL	190.12±5.34###+++	180.47±8.94###+++	220.36±15.43###+
GTE500+CDL	145.41±6.97###+++	150.01±4.63###+++	191.17±10.15###+++

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

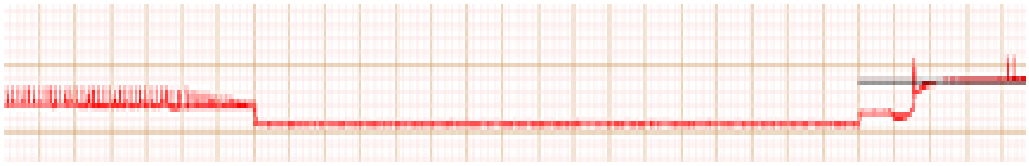
TABLE 3. EFFECTS ON PERCENTAGE RECOVERY IN TERMS OF HEART RATE AND DEVELOPED TENSION AGAINST IRI

GROUP	% RECOVERY IN TERMS OF HEART RATE	% RECOVERY IN TERMS OF DEVELOPED TENSION
IRI	50.01±0.67	30.89±0.67
CDL	72.33±0.55###	69.78±0.78###
GTE100	60.59±0.43###	48.77±O.42###
GTE500	67.24±0.71###	55.67±0.95###
GTE100+CDL	82.68±0.39###+++	78.23±0.78###++
GTE500+CDL	90.45±0.58###+++	87.76±0.43###++

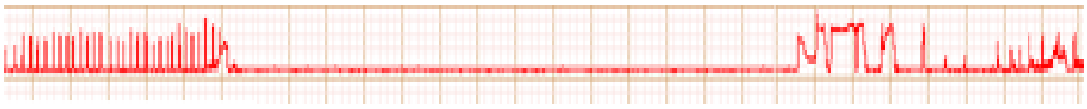
VALUES ARE EXPRESSED AS MEAN±SEM. N=6. ###P<0.001 WHEN COMPARED WITH IRI. +P<0.05, +++P<0.001 WHEN COMPARED WITH CDL.

FIGURE 1. PHYSIOGRAPH RECORDING

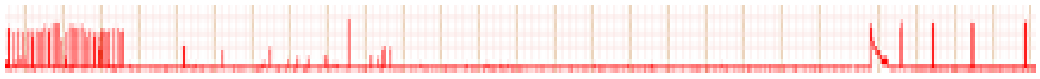
PHYSIOGRAPH SHOWING THE EFFECT OF DRUG ON ISCHEMIA REPERFUSION INDUCED MYOCARDIAL DAMAGE



Tracing on physiograph of normal heart (IRI control) at an speed of 12.8 sec /10 mm.



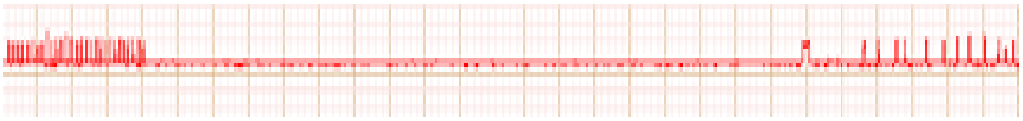
Tracing on physiograph of Carvedilol (10mg/kg) treated heart at a speed of 12.8 sec /10 mm.



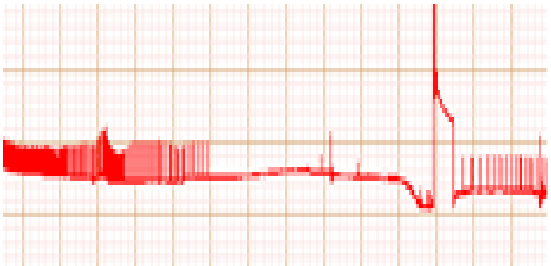
Tracing on physiograph of GTE 100 mg/kg treated heart at a speed of 12.8 sec /10 mm.

FIGURE 2. PHYSIOGRAPH RECORDING

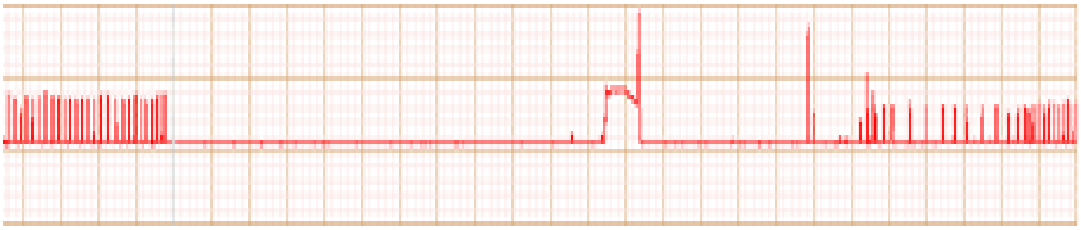
PHYSIOGRAPH SHOWING THE EFFECT OF DRUG ON ISCHEMIA REPERFUSION INDUCED MYOCARDIAL DAMAGE



Tracing on physiograph of GTE 500 mg/kg treated heart, at a speed of 12.8 sec/10 mm.



Tracing on physiograph of GTE 100 mg/kg + CDL 10 mg/kg treated heart at an speed of 12.8 sec/10 mm.



Tracing on physiograph of GTE 500mg/kg + CDL 10mg/kg treated heart at an speed of 12.8 sec/10 mm.

DISCUSSION

In ischemia supply of oxygen to the cells will be reduced. Oxidative phosphorylation in the cells will be decreased. Then cells will shift to glycolysis. Anerobic metabolism will start and there will be decrease in cellular ATP. Hence there will be increase in AMP. All these things will lead to glycolysis. This will lead to accumulation of lactic acid mainly. Hence intracellular pH will go down which will affect the activity of many cellular enzymes [26].

After myocardial ischemia there will be stopping of aerobic respiration. When there is no aerobic respiration there wont be any adenosine triphosphate production. Increase in lactic acid will be seen [27].

Reperfusion will cause disturbance in various ions. In cytosol it will cause calcium overload. Then there will be generation of reactive oxygen species. These will cause damage to the cells [28].

Also reperfusion will cause harmful changes in coronary arteries and myocardial tissues. This will result in cardiac dysfunction. This dysfunction will be called as ischemia reperfusion injury. This will cause various other diseases like angina pectoris [29], myocardial infraction [30] etc.

The main result of ischemic reperfusion is that it will cause contractile dysfunction and derangement of blood flow [31]. If this goes on for a long duration of time then there will be various other problems such as decrease in ATP, collagen matrix will be damaged and above derangements due to reperfusion[32].

As described earlier that ischemia will cause reduction in oxidative phosphorylation and production of energy rich phosphates it will also cause cellular swelling. Cellular swelling will happen due to increase in entry of water and sodium into the cells [33].

The various reactive oxygen species which would be produced are hydrogen peroxide, hydrogen radical, superoxide anion [34,35,36]

Green tea cause improves the above mentioned derangements. High dose of green tea extract is more effective than its low dose. It shows synergistic action with carvedilol. If one is consuming green tea along with carvedilol, then the dose of carvedilol can be reduced. By doing this it will lessen the toxic side effects of carvedilol in the body.

CONCLUSION

In OPPTS toxicity study, green tea extract failed to show any signs of toxicity. It improved the conditions of the animals suffering from ischemia reperfusion induced myocardial damage and showed synergistic action with carvedilol.

ACKNOWLEDGEMENT

The authors like to thank SHREE DEVI EDUCATION TRUST (R), MANGALORE for giving the necessary facilities for carrying out the research work.

REFERENCES

1. Berman AF. Herb–drug interactions. Lancet 2000;355(9198):134-8.
2. Law MEW, Wang XL, Nawano M, Granner DK. Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. J Biol Chem 2002;277(38):34933–40.
3. Information on carvedilol. Available from: http://www.medicinenet.com/carvedilol
4. Information on carvedilol. Available from: http://www.drugs.com/mtm/carvedilol.html
5. Information on carvedilol. Available from: en.wikipedia.org/wiki/Carvedilol
6. Information on carvedilol. Available from: http://www.patientsville.com/medication
7. Information on carvedilol. Available from: http://newsletters.healthscout.com
8. Information on carvedilol. Available from: http://www.druglib.com/druginfo/coreg/
9. Information on carvedilol. Available from: http://www.accessdata.fda.gov
10. Information on carvedilol. Available from: www.carvedilol-side-effect-smechanism.com
11. Information on carvedilol. Available from: us.gsk.com/products/assets/us_coreg.pdf
12. Information on carvedilol. Available from: www.uk.gsk.com.sr
13. Information on carvedilol. Available from: www.congestiveheartfailure.emedtv.com
14. Yamada H, Watanabe H. Tea polyphenols in preventing cardiovascular diseases. Cardio Res. 2007; 73: 439-440.
15. Kuriyama S. Green tea consumption and prevention of coronary artery disease. Official journal of the Japanese Circulation Society. www.j-circ.jp.
16. Pandey S. Tea:Effects on cardiovascular system, blood pressure, eeg and many other systems of human beings. IJPSRR. 2010; 2(1): 31.
17. Antonello M. Prevension of hypertension, cardiovascular damage and endothelial dysfunction with green tea extracts. AJH. 2007; 20: 1321-1328.
18. Kokate CK. Practical Pharmacognosy. 3rd ed. New Delhi : VPBN 1991: 107-11.
19. Acute oral toxicity study. Available from: www.epa.gov/opptsfrs, 2008.
20. Spring MS, Grotthus B, Szelag A, Isolated heart perfusion according to Langendorff-Still viable in the new millennium. J Pharmacol Toxicol Meth 55; 113-26, 2007.
21. Asdaq SMB, Inamdar MN. Pharmacodynamic interaction of garlic with captopril in Ischemia-reperfusion induced myocardial damage in rats. Pharmacologyonline. 2008;2:875-88.

22. S.K Kulkarni. Handbook of experimental pharmacology. New Delhi:Vallabh Prakashan 2010.
23. Nandi A and Chatterjee I B. Assay of superoxide dismutase activity in animal tissues. J. Biosci,1988;13:305-315.
24. Sinha A K. Colorimetric assay of catalase. Anal Biochem,1972;47:389-394.
25. Sedlakova E, Racz O, Lovasova E, Benacka R, Kurpas M, Chmelarova A. Markers of oxidative stress in acute myocardial infarction treated by percutaneous coronary intervention. Cent Eur J Med 2009;4: 26-31.
26. Robbins In :Basis of disease. India: Harcourt (India) Private Limited;2000.p.15-6.
27. Robbins. IHD, In :Pathologic Basis of Disease. India: Harcourt (India) Private Limited;2000.p.571-87.
28. Jennings RB, Schaper J, Hill ML. Effect of reperfusion late in the phase of reversible ischemic injury: changes in cell volume, electrolytes, metabolites, and ultrastructure. Circ Res.1985;56:262-78.
29. Verma S., Paul W.M., Fedak R.D., Weisel M.D., Butany J., Rao V., Maitland A., Li R.K., Dhillon B., Yau T.M. "Fundamentals of Reperfusion Injury for the Clinical Cardiologist". *Circulation* 2002; 105: 2332-6.
30. McDonough J.L., Arrell D.K., Van Eyk J.E. "Troponin I Degradation and Covalent Complex Formation Accompanies Myocardial Ischemia/Reperfusion Injury". Circ Res 1999; 84: 9-20.
31. Bolli R. Mechanism of myocardial "stunning." Circulation, 1990; 82:723-38.
32. Ferrari R., Visioli O. "Particular outcomes of myocardial ischaemia: Stunning and hibernation". Pharmacol Res 1995; 31:235-41.
33. Buja L.M."Myocardial ischemia and reperfusion injury".Cardiovasc Pathol 2005;14:170-5.
34. Touyz R.M., Schiffrin E.L. "Ang II-stimulated superoxide production is mediated via phospholipase D in human vascular smooth muscle cells". Hypertension 1999; 34:976-982.
35. Rao G.N., Berk B.C. "Active oxygen species stimulated vascular smooth muscle cell growth and protooncogene expression". Circ Res 1992; 70:593-599.
36. Zafari A.M., Ushio-Fukai M., Yin Q., Shah A., Harrison D.G., Taylor W.R., Griendling K.K. "Role of NADH/NADPH oxidase-derived H2O2 in angiotension II-induced vascular hypertrophy". Hypertension 1998; 32:488-495.