Ahmad Amanullah et al. / Journal of Pharmacy Research 2015,9(9),581-587

Research Article
ISSN: 0974-6943

Available online through
www.jprsolutions.info

Evaluation of Combinational Therapy of Resveratrol and Black Tea Extract on Doxorubicin-induced Myocardial Infarction in Wistar Rats

Ahmad Amanullah, Rustam Ekbbal and Syed Ehtaishamul Haque*
Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi-110062, India

Received on:28-07-2015; Revised on: 17-08-2015; Accepted on: 22-09-2015

ABSTRACT

Background: Cardiovascular and cancer problem is increasing day by day and causing untimely deaths worldwide. A continuous research is going on to develop new cardioprotective and anticancer drugs which are safe, economical and effective. Doxorubicin is a potent anticancer drug with restricted use due to cardio toxicity that it causes as side effect. Thus, any drug which will reduce its toxicity will be a boon for cancer patient. Herbal drugs are emerging as potent targets for research because they are cheap, locally available; having least reported side effects and generally being the part of our food culture. In view of this we have tried to evaluate the cardioprotective potential of resveratrol and black tea extract alone and in combination on the doxorubicin-induced myocardial infarction in rats. **Methods:** Thirty rats of Wistar albino strain were taken and divided into Control, Doxorubicin (DOX), Black Tea + DOX, Resveratrol + DOX, Black Tea + Resveratrol and Black Tea + Resveratrol + DOX treated groups. Various biochemical parameters were assessed to evaluate their antioxidant property and myocardial membrane integrity. Effect of these neutraceuticals on cellular architecture was also assessed by histopathological studies. **Results and Discussions:** Biochemical and histopathological investigations clearly showed the reversal of all the parameters towards normal indicating improvement in antioxidant status and reduction in cardiac damage. **Conclusion:** All the test drugs administered groups ; black tea-treated, resveratrol-treated and their combination-treated groups, showed cardio protection but the combination-treated group proved to be a better cardioprotective agent than the two drugs alone.

KEYWORDS: Resveratrol, black tea, doxorubicin, myocardial infarction, antioxidant.

1. INTRODUCTION

Heart disease is one of the fatal diseases reported globally which includes myocardial infarction, arteriosclerosis, hypertension, congestive heart failure, coronary heart disease, arrhythmia, hypertrophy, ventricular fibrillation, ventricular tachycardia and strokes [1-3] . According to the statistics given by WHO 2012 [4], about 17.5 million people around the globe were died due to cardiovascular diseases, which forms about one-third of the total global deaths. It is predicted that heart disease and stoke will become the leading cause of deaths and disability world-wide by the year 2020. The number of fatalities is projected to increase more than 24 million by the year 2030.The developing country like India is struggling to manage the impact of infectious disease along with the growing burden of non-communicable diseases such as myocardial infarction [5]. Moreover, it is painful and of serious concern to realize that myocardial infarction (MI) in India occurs 10 to 15 years earlier as compared to west [6]. Current projections suggest a highly notorious, but a real fact that by the year 2020 India will have the largest cardiovascular disease burden in the World [7].

***Corresponding author.**
Dr. Syed Ehtaishamul Haque
Department of Pharmacology,
Faculty of Pharmacy,
Jamia Hamdard, New Delhi 11 0062, India

Oxidative stress, associated with an increased formation of reactive oxygen species (ROS), is implicated in the etiopathogenesis of a variety of human diseases including cardiovascular diseases (CVDs) like atherosclerosis, myocardial ischemia-reperfusion injury (MI/RI) and cardiomyopathy [8]. Oxidative stress modifies lipids and proteins, leading to lipid peroxidation and oxidation of thiol groups, which consequently alter membrane permeability and configuration and produce functional alteration of various cellular proteins. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been widely postulated to contribute to the cardiotoxic properties of many drugs including anthracyclines [9].

Doxorubicin (DOX) also called as adriamycin, an anthracycline antibiotic, is a potent broad –spectrum chemotherapeutic agent that is highly effective in treating patients with acute lymphoblastic leukemia, Hodgkin's lymphoma, aggressive non-Hodgkin's lymphomas and many solid tumors [10]. However, the clinical use of this drug has been seriously limited by undesirable side effects especially dose-depen-

dent myocardial injury leading to potentially lethal congestive heart failure [11]. If the cumulative dose of DOX exceeds 550 mg/m^2 body surface, the risk of developing cardiomyopathy, cardiac dilation, and finally decompensated heart failure sharply increases [12]. Thus any drug which will reduce the toxicity will prove to be a boon for the cancer patient.

A wide variety of drug treatments are available for acute MI including thrombolytic, anticoagulants, ß- blockers, nitrates, anti platelets and ACE inhibitors. Clot dissolving drugs are also available for the treatment of MI. However, clot dissolving drugs often lead to ischemia reperfusion (I/R) injury in heart which occurs due to burst of free radicals produced during reoxygenation of tissue that has become hypoxic [13]. Hence there is a need of developing newer therapeutic strategies that aim to limit the infarct size and enhance the myocardial function. People now-a-days are paying lot of attention towards the herbal drugs which are economical, safe, effective and part of day to day life. These nutraceuticals have shown to possess antioxidant, anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic properties which adds to their popularity as potent candidate for research studies. Thus their uses are rapidly emerging as effective alternative or adjuncts for modern medicine.

Black tea (*Camellia sinensis*) is the most widely used beverage and also one of the main sources of polyphenols [14], so there is a realization that black tea can be investigated for their beneficial effects on health. Black tea is now receiving considerable scientific attention unfolding several health promoting effects like antihypertensive [15], anti-cancer [16], anti–inflammatory [17], antioxidative [18], antiulcer [19], antidiabetic [20], antimutagenic [21], anticlastogenic [22], antiproliferative [23], antipathogenic [24] and antiparkinsonian [25].

Resveratrol (3, 5, 4´-*trans*-trihydroxy stilbene) belongs to the stilbene family and was detected for the first time in *Vitis vinifera* grapevines [26]. It was synthesized in 1992 from the leaves by fungal infection or UV light [27]. Resveratrol has been reported to have antioxidant and apoptotic activities [28]. It is a traditionally used medicinal herb which has been reported to increase the life span of yeast [29], mice, insulin sensitivity [30]. It has also been reported to prevent cancer [31], play neuroprotective role in Alzheimer's disease [32], extends renal protection [33-34], prevent atherosclerosis, hyperlipidemia and inflammation [35]. Although black tea and resveratrol has already been investigated against various indications, their combinational studies have not been performed extensively. They have been reported to possess anti-tumor effect [36] and anti-obesity effect so far [37]. However their cardioprotective role in combination is not explored. Thus we tried to investigate the combinational effect of these two drugs on doxorubicin induced myocardial infarction in Wistar albino rats.

2. MATERIALS AND METHODS

2.1. EXPERIMENTAL FRAMEWORK:

The experimental study was carried out under standard laboratory conditions in adult Wistar albino rats of female sex (150-200 g), procured from Central Animal House Facility of Hamdard University, New Delhi. The rats were maintained on animal diet obtained from Ashirwad Laboratory Animal Feed manufactured by Ashirwad Industries, Mohali, Punjab and water *ad -libitum.* The protocol (No. 1110) was approved by the institutional animal ethics committee of Hamdard University, New Delhi.

Animals were divided into 6 groups; each group consisting of 5 animals. The animal groups and their treatment schedules were as follow.

Table 1: Experimental plan

Groups (n = 5)		Treatment Schedule
I	Control	D.W (2 ml/kg, p.o) once a day for 30 days + Normal saline (1 ml/kg, i.p) once on 29th day.
II	DOX	D.W (2 ml/kg, p.o) once a day for 30 days + DOX (20 mg/kg, i.p) once on 29th day.
III.	BTE + DOX	20 % BTE (2 ml/kg, p.o) once a day for 30 days + DOX (20 mg/kg, i.p) once on 29th day.
IV	RESV + DOX	Resv (20 mg/kg, p.o) once a day for 30 days + DOX (20 mg/kg, i.p) once on 29th day.
V	BTE + RESV	20 % BTE (2 ml/kg, p.o) + Resv (20 mg/kg, p.o) once a day for 30 days
VI	BTE+ RESV+DOX	20 % BTE (2 ml/kg, p.o) + Resv (20 mg/kg, p.o) once a day for 30 days + DOX (20 mg/kg, i.p) once on 29th day.

Animals were sacrificed on 31st day using ether anesthesia. Blood samples (2-3 ml) were collected in sterile centrifuged tubes under light ether anesthesia, exactly 48 hours of the last dose of DOX from the rat tail vein and serum was prepared for the estimation of various biochemical parameters. Hearts from the sacrificed animals were removed and weighed. Heart of rats from each group was homogenized for biochemical estimations in myocardium and some of them were fixed in 10% formalin for histopathological investigation.

2.2. DIAGNOSTIC ESTIMATIONS

Following biochemical estimations were performed in serum:

Lactate Dehydrogenase (LDH) [38]

Creatine Kinase MB (CK-MB) [39]
Total Antioxidant Capacity (TAC) [40]

Biochemical estimations in cardiac tissue:
Tissue Glutathione (reduced GSH level) [41]
Thiobarbituric Acid Reactive Substances (TBARS)[42]

2.3. STATISTICAL ANALYSIS

Data were expressed as mean ± SEM (standard error mean) of five rats per group. Means were compared by one way analysis of variance (ANOVA) with post hoc analysis. The Tukey-Kramer post hoc test was applied to identify the significance among different groups. $P < 0.05$ was considered statistically significant. Graph Pad software, Inc. version (5.01) was used for statistical analysis.

2.4. HISTOPATHOLOGICAL STUDIES

Method: Haematoxylin

Procedure:
After sacrificing the animals, hearts were removed, washed in ice-cold normal saline and preserved in 10 % formalin. Tissues were then stained by eosin-haematoxylin stains and their paraffin sections were studied to determine the extent of tissue damage by the free radicals and the influence of the test drugs.

3. RESULTS:

3.1. Effect of resveratrol and black tea extract on H.W/B.W ratio

The H.W/B.W ratio of Group II (DOX) was found to be significantly low ($p < 0.001$) when compared with Group I (CONTROL). Groups III (BTE + DOX), Group IV (RESV + DOX) and Group VI (BTE + RESV + DOX), showed significant increase ($p < 0.001$) in H.W/B.W ratio when compared with the toxic group (Group II). Group V (BTE + RESV), which was drug alone treated group, however, showed no significant changes as compared to the control group (Table 2).

Table 2: Observations of the H.W/B.W ratio of different groups

Group	Treatment	H.W (g)	B.W (g)	H.W/B.W ratio
I	CONTROL	1.21 ± 0.03	255.90 ± 5.39	0.0047 ± 0.0001
II	DOX	0.32 ± 0.02	162.50 ± 3.35	0.0020 ± 0.0001$^{###}$
III	BTE + DOX	0.64 ± 0.04	205.00 ± 7.19	0.0031 ± 0.0001*
IV	RESV + DOX	0.60 ± 0.03	209.16 ± 4.36	0.0029 ± 0.0001*
V	BTE + RESV	1.09 ± 0.04	262.50 ± 4.96	0.0042 ± 0.0001
VI	BTE+ RESV + DOX	0.71 ± 0.04	221.67 ± 5.72	0.0032 ± 0.0001*

H.W, heart weight; B.W, body weight; DOX, doxorubicin (20 mg/kg, i.p once on 29th day); BTE, black tea extract (2 ml/kg /day/oral of 20 % BTE) + DOX, doxorubicin (20 mg/kg, i.p once on 29th day); RESV, resveratrol (20 mg/kg /day/oral) + DOX, doxorubicin (20 mg/kg, i.p once on 29th day); BTE + RESV, black tea extract (2 ml/kg/day/oral/ of 20 % BTE) + resveratrol (20 mg/kg /day/oral); BTE+ RESV + DOX , black tea extract (2 ml/kg /day/oral of 20 % BTE) + RESV, Resveratrol (20 mg/kg /day/ oral) + doxorubicin (20 mg/kg, i.p once on 29th day). Data represent mean ± SEM of five rats per group. $^{###}p < 0.001$ vs. Control and $^{*}p < 0.001$ vs. DOX.

3.2. Effect of resveratrol and black tea extract on LDH activity:

The LDH activity of Group II (DOX) was found to be significantly high ($p < 0.001$) when compared with Group I (CONTROL). Groups III (BTE + DOX), Group IV (RESV + DOX) and Group VI (BTE + RESV + DOX), which were drug treated groups, showed significant decrease ($p < 0.001$) in LDH activity when compared with the toxic group (Group II). Group V (BTE + RESV), which was drug alone treated group, however, showed no significant changes as compared to the control group (Table 3).

Table 3: Biochemical observations of serum parameters in different groups

Group	Treatment	LDH (IU/L)	CK-MB (IU/L)	TAC (µM/L)
I	CONTROL	61.61 ± 3.04	69.21 ± 6.09	1.58 ± 0.02
II	DOX	164.58 ± 6.10$^{###}$	192.43 ± 3.70$^{###}$	0.87 ± 0.02$^{###}$
III	BTE + DOX	121.25 ± 2.52*	146.86 ± 4.34*	1.16 ± 0.03*
IV	RESV+ DOX	123.22 ± 5.80*	156.98 ± 4.34*	1.20 ± 0.03*
V	BTE + RESV	67.52 ± 3.67	74.27 ± 4.27	1.79 ± 0.03
VI	BTE + RESV + DOX	97.90 ± 2.82$^{*\$}$	116.47 ± 4.34$^{*?}$	1.46 ± 0.03$^{*\Delta}$

LDH, lactate dehydrogenase; CK-MB, creatine kinase MB isozyme; TAC, total antioxidant capacity; DOX, doxorubicin (20 mg/kg, i.p once on 29th day); BTE, black tea extract (2 ml/kg /day/oral of 20 % BTE) + DOX, doxorubicin (20 mg/kg, i.p once on 29th day); RESV, resveratrol (20 mg/kg /day/oral) + DOX, doxorubicin (20 mg/kg, i.p once on 29th day); BTE + RESV, black tea extract (2 ml/kg/day/oral/ of 20 % BTE) + resveratrol (20 mg/kg /day/oral); BTE + RESV + DOX, black tea extract (2 ml/kg /day/oral of 20 % BTE) + RESV, Resveratrol (20 mg/kg / day/ oral) + doxorubicin (20 mg/kg, i.p once on 29th day). Data represent mean ± SEM of five rats per group.$^{###}p < 0.001$ vs. Control,$^{*}p < 0.001$ vs. DOX, $^{?}p < 0.001$, $^{\$}p < 0.01$ vs. BTE + DOX & RESV + DOX.

3.3. Effect of resveratrol and black tea extract on CK-MB activity:

The CK-MB activity of Group II (DOX) was found to be significantly high ($p < 0.001$) when compared with Group I (CONTROL). Groups III

(BTE + DOX), Group IV (RESV + DOX) and Group VI (BTE + RESV + DOX), which were drug treated groups, showed significant decrease ($p < 0.001$) in CK-MB activity when compared with the toxic group (Group II). Group V (BTE + RESV), which was drug alone treated group, however, showed no significant changes as compared to the control group (Table 3).

3.4. Effect of resveratrol and black tea extract on total antioxidant capacity (TAC) activity:

The total antioxidant capacity (TAC) of Group II (DOX) was found to be significantly low ($p < 0.001$) when compared with Group I (CONTROL). Groups III (BTE + DOX), Group IV (RESV + DOX) and Group VI (BTE + RESV + DOX) showed significant increase ($p < 0.001$) in total antioxidant capacity when compared with the toxic group (Group II). However, Group V (BTE + RESV) alone, showed no significant changes as compared to the control group (Table 3).

3.5. Effect of resveratrol and black tea extract on TBARS concentration:

The TBARS concentration of Group II (DOX) was found to be significantly high ($p < 0.001$) when compared with Group I (CONTROL). Group III (BTE + DOX), Group IV (RESV + DOX) and Group VI (BTE + RESV + DOX) showed significant decrease ($p < 0.001$) in TBARS concentration when compared with the toxic group (Group II). Group V (BTE + RESV) however, showed no significant changes as compared to the control group (Table 4).

Table 4: Biochemical observation in the heart tissues of different groups

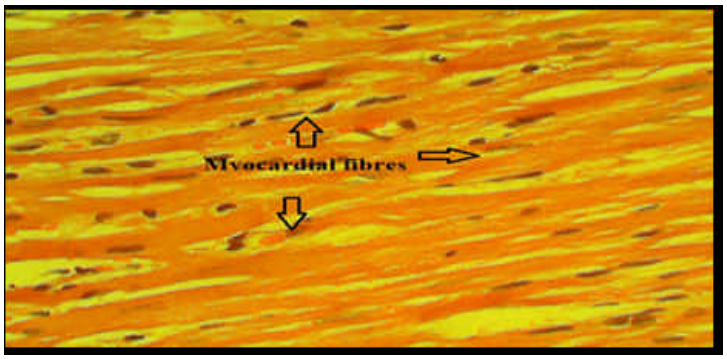
Group	Treatment	TBARS (µM/L)	Tissue GSH (µM/L)
I	Control	0.25 ± 0.01	15.83 ± 0.80
II	DOX	0.68 ± 0.00$^{###}$	6.53 ± 0.72$^{###}$
III	BTE + DOX	0.49 ± 0.02*	11.23 ± 0.72*
IV	RESV + DOX	0.48 ± 0.01*	10.45 ± 0.65*
V	BTE + RESV	0.26 ± 0.00	14.47 ± 0.64
VI	BTE+ RESV +DOX	0.40 ± 0.01$^{*?\$}$	10.33 ± 0.48*

TBARS, thiobarbituric acid reactive substances; GSH, glutathione; DOX, doxorubicin (20 mg/kg, i.p once on 29th day); BTE, black tea extract (2 ml/kg /day/oral of 20 % BTE) + DOX, doxorubicin (20 mg/kg, i.p once on 29th day); RESV, resveratrol (20 mg/kg /day/oral) + DOX, doxorubicin (20 mg/kg, i.p once on 29th day); BTE + RESV, black tea extract (2 ml/kg/day/oral/ of 20 % BTE) + resveratrol (20 mg/kg /day/ oral); BTE+ RESV + DOX , black tea extract (2 ml/kg /day/oral of 20 % BTE) + RESV, Resveratrol (20 mg/kg /day/ oral) + doxorubicin (20 mg/kg, i.p once on 29th day). Data represent mean ± SEM of five rats per group. $^{###}p < 0.001$ vs. Control, $^{*}p < 0.001$ vs. DOX, $^{?}p < 0.001$ vs. BTE + DOX & $^{\$}p < 0.01$ vs. RESV + DOX.

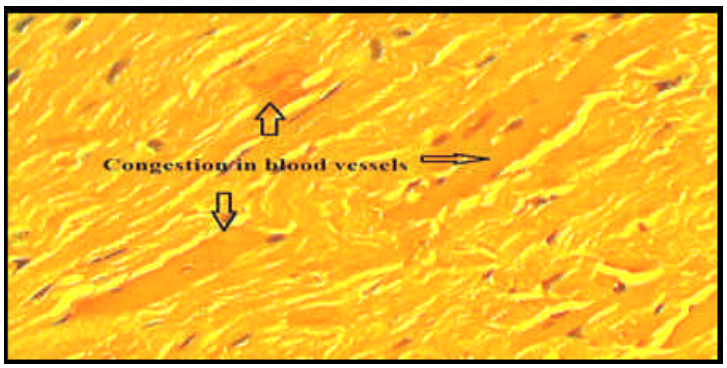
3.6. Effect of resveratrol and black tea extract on tissue GSH levels:

The tissue GSH levels of Group II (DOX) was found to be significantly low ($p < 0.001$) when compared with Group I (CONTROL). Group III (BTE + DOX), Group IV (RESV + DOX) and Group VI (BTE + RESV + DOX) showed significant increase ($p < 0.001$) in tissue GSH levels when compared with the toxic group (Group II). Group V (BTE + RESV), however, showed no significant changes as compared to the control group (Table 4).

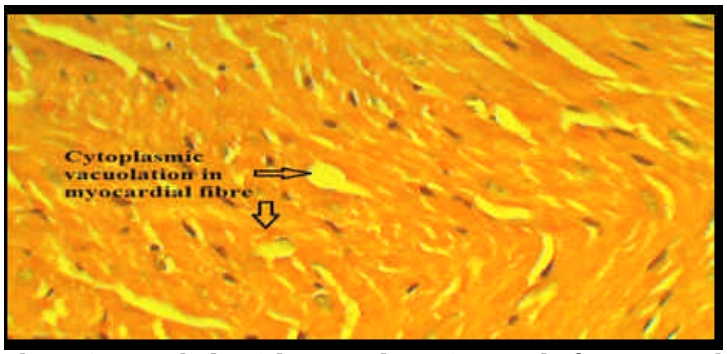
3.7. HISTOPATHOLOGICAL STUDIES



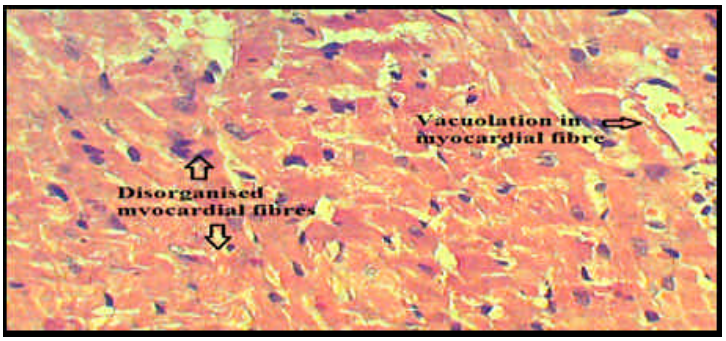
Photomicrograph 1: High power photomicrograph of control animal (Group I) showing regular and well oriented branching cardiac muscle fibres with flat & oval nuclei HE × 400).



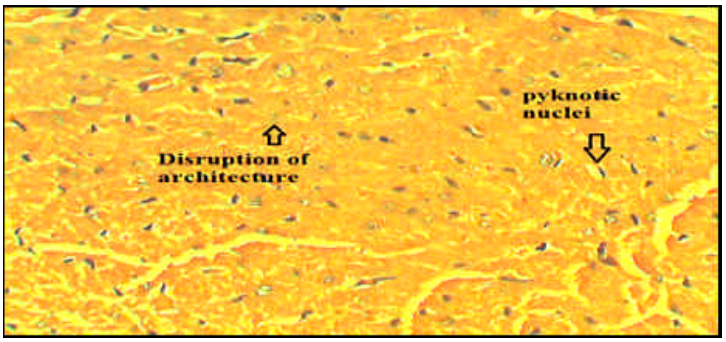
Photomicrograph 2a: High power photomicrograph of DOX treated animal (Group II) showing area of myocytolysis and congestion in blood vessels HE × 400).



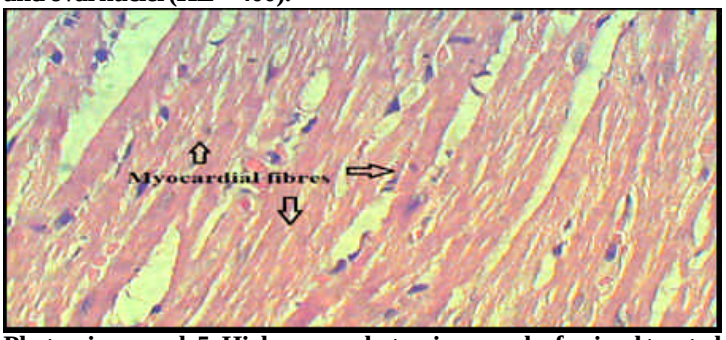
Photomicrograph 2b: High power photomicrograph of DOX treated animal (Group II) showing cytoplasmic vacuolation and presence of central & oval nuclei (HE × 400).



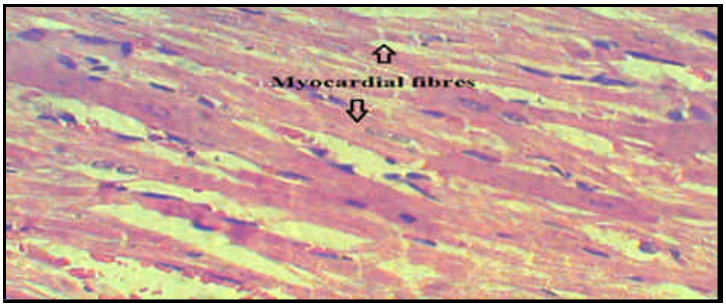
Photomicrograph 3: High power photomicrograph of animal treated with BTE + DOX (Group III); showing mild disorganized myocardial fibres, congestion of blood capillaries, vacuolation and some flattened and oval nuclei (HE × 400).



Photomicrograph 4: High power photomicrograph of animal treated with RESV + DOX (Group IV) showing some disruption of architecture, congestion of blood capillaries and presence of some pyknotic and oval nuclei (HE × 400).



Photomicrograph 5: High power photomicrograph of animal treated with BTE + RESV, (Group V) showing normal, regular and well oriented morphology of myocardial fibres (HE × 400).



Photomicrograph 6: High power photomicrograph of animal treated with BTE+ RESV + DOX, (group VI) showing cardiac muscle fibres of normal shape, size and configuration with minimal disorganization of myocardial fibres and single perinuclear edema (HE × 400).

4. DISCUSSION

Doxorubicin (DOX) also called as adriamycin, an anthracycline antibiotic, is a potent broad –spectrum chemotherapeutic agent that is highly effective in treating patients with acute lymphoblastic leukemia, Hodgkin's lymphoma, aggressive non-Hodgkin's lymphomas and many solid tumors [10], but its clinical use has been seriously limited by undesirable side effects especially dose-dependent myocardial injury, leading to potentially lethal congestive heart failure [11]. The oxidative stress produced by doxorubicin causes cellular damages and leads to decrease in the antioxidant enzymes and various other biochemical changes [8, 9].

In our study when we have treated the rats with doxorubicin (20 mg/kg i.p.), we also found a decrease in the antioxidant defense status. Reduced GSH & TAC level, which shows the cellular antioxidant defense status of the heart, got reduced under oxidative stress created by DOX treatment. RESV, BTE and their combination on administration significantly restored their level showing augmentation of the antioxidant defense. This restoration however, was better seen with the combination treatment when compared with the BTE + DOX and RESV + DOX groups.

Oxidative stress modifies lipids and proteins, leading to lipid peroxidation and oxidation of thiol groups, consequently altering membrane permeability and configuration and producing function alteration of various cellular proteins. In our case we also observed an increase in lipid peroxidation (TBARS) due to generation of reactive oxygen species (ROS) by DOX which was significantly reverted on treatment with our test drugs. Among the drug treated groups the combination group registered better effect than the two drugs alone in reducing lipid peroxidation.

The myocardial enzymes like LDH, CK-MB etc. increases due to leakage of the myocardium and their concentration become more in serum. In our case we have observed the same when we treated our rats with DOX. Our drug RESV, BTE and their combination decreased the level of these enzymes significantly showing potential role in protecting the integrity of myocardial membrane. Combination group however, showed better result than the two drugs alone.

Under DOX-induced oxidative stress we also noticed significant changes in the cellular architecture of heart; like vacuolization, myocarditis and congestion of muscle fibres and pyknotic nuclei. However, when we have treated the rats with RESV, BTE and their combination we found restoration of cellular architecture to normal.

5. CONCLUSION

Thus considering all the biochemical and histopathological results, we came to this conclusion that Resveratrol, BTE and their combina-

tion considerably reduced the serum marker enzymes (LDH, CK-MB), TBARS level; improved membrane integrity and boosted the antioxidant defense of the body by increasing total antioxidant capacity and reduced glutathione level. The cellular architecture obtained from histopathological studies also substantiated the above finding and strongly indicated towards the effective cardioprotective role of resveratrol and black tea extract. The combination of these two drugs, however shown better effects than the single drug.

6 REFERENCES

1. Miller, L.W., Missov, E.D. Epidemiology of heart failure. Cardiol. Clin. 2001; 19: 547-555.
2. Lopez, F.A., Casado, S. Heart failure, redox alterations and endothelial dysfunction. Hypertension. 2001; 38: 1400-1405.
3. Bagchi, D., Das, D.K., Engelman, R.R., Prasad M.R., Subramanian, R. Polymorphonuclear leucocytes as potential source of free radical in the ischemic repurfused myocardium. Eur. Heart J. 1990; 11: 800-13.
4. World Health Organisation. Global health observatory data death from cardiovascular disease. 2012.
5. Farvin, K.H.S., Anandan, R., Kumar, S.H.S., Shiny, K.S., Shankar, T.T., Thankappan, T.K. Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. Pharmacol.Res. 2004; 50: 231-236.
6. Dhandapani, N., Ganesan, B., Anandan, R., Jeyakumar, R., Rajaprabhu, D., Ezhilan, A. Synergistic effects of squalene and polyunsaturated fatty acid concentrate on lipid peroxidation and antioxidant status in isoprenaline-induced myocardial infarction in rats. African Journal of biotechnology 2007; 6 (8): 1021-1027.
7. Yusuf, S., Reddy, S., Ounpu, S. Global burden of cardiovascular diseases (part-1) general considerations, the epidemiological transition, risk factors and impact of urbanization. Circulation 2001; 104: 2746-2753.
8. Kaul, N., Siveski-Iliskovic, N., Hill, M., Slezak, J., Singal, P.K. Free radicals and the heart. J. Pharmacol. Toxicol. Meth. 1993; 30: 55-57.
9. Doroshow, J.H. Effect of anthracyclin antibiotics on oxygen radical formation in rat heart. Cancer Res. 1983; 43: 460-472.
10. Wallace, K.B. Doxorubicin-induced cardiac mitochondriopathy. Pharmacology and toxicology 2003; 93: 105-115.
11. Singal, P.K., Li, T., Kumar, D., Danelisen, I., Iiskovic, N. Adriamycin induced heart failure; Mechanism and modulation. Mol. Cell Biochem. 2000; 207: 77-85.
12. Minotti, G., Menna, P., Salvatorelli, E., et al. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev. 2004; 56: 185–229.
13. Maxwell, S.R.J., Lip, G.Y.H. Reperfusion injury; A review of the pathophysiology, clinical manifestation and therapeutic option. Int. J. Cardiol. 1997; 58: 95-117.
14. Rechner, A.R., Wagner, E., Van Buren, L., Van De Put, F., Wiseman, S., Rice-Evans, C.A. Black tea represents a major source of dietary phenolics among regular tea drinkers. Free Radiac Res. 2002; 36(10): 1127-1135
15. Hadgson, J.M., Puddey, I.B, Burke, V., Beilin, L.J., Jordan, N. Effects on blood pressure of drinking green and black tea. J Hypertens 1999; 457-63.
16. Das, M., Chaudhary, T., Goswami, S.K., et al. Studies with black tea and its constituents on leukemic cells and cell lines' Expr Clan Cancer RES. 2002; 21: 563-568.
17. Nag Chaudhary, A.K, Karmakar, S., Roy, D., Pal, S., Pal, M., Sen, T. Anti inflammatory activity of Indian black tea (Sikkim variety). Pharmacol Res. 2005; 51(2):169-175.
18. Luczaj, W., Skrzydlewska, E. Antioxidative properties of black tea. Prev Med. 2005; 40(6): 910-918.
19. Maity, S., Veda-Siromoni, J.R., Gangulay, D.K. Antiulcer effect of the hot water extract of black tea (Camellia sinensis). J Ethnopharmacol 1995; 46: 167-174.
20. Gomes, A.,Vedasiromoni. J.R., Das, M., Sharma, R.M., Ganguly, D.K. Antihyperglycemic effect of black tea *(Camellia sinensis*) in rat. Ethnopharmacol 1995; 45: 223-226.
21. Halder, B., Pramanick, S., Mukhopadhyay, S., Giri, A.K. Inhibition of benzo(a)pyrene-induced mutagenicity and genotoxicity by black tea polyphenols theaflavins and thearubugins in multiple test systems. Food Chem Toxicol 2005; 43:591-7.
22. Halder, B., Pramanick, S., Mukhopadhyay, S., Giri, A.K. Anticlastogenic effects of black tea polyphenols theaflavins and thearubigins in human lymphocytes in vitro. Toxicol in vitro 2006; 20:608-13.
23. Chandra Mohan, K.V.P., Devaraj, H., Prathiba, D., Hara, Y., Nagini, S. Antiproliferative and apoptosis inducing effect of lactoferrin and black tea polyphenol combination hamster pouch carcinogenesis. Biochem Biophys Acta. 2006; www.sciencedirect.com.
24. Wang C, Li. Research progress on property and application of theaflavins. Afr J Biotechnol 2006; 5(3):213-18.
25. Chaturvedi, R.K., Shukla, S., Seth, K., Chauhan, S., Sinha, C., Shukla, Y., Agarwal, A.K. Neuroprotective and neurorescue effect of black tea extract in 6-hydroxydopaminelesioned rat model of Parkinson's disease. Neurobiol Dis 2006; 22(2):421-434.
26. Langcake, P., and Pryce, R.J. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. Physiol. Plant Pathol. 1976; 9: 77-86.
27. Siemann, E. H., and Creasy, L. L. Concentration of the phy-

toalexin resveratrol in wine. Am. J. Enol. Vitic. 1992; 43: 49-52.
28. Yu-Jun, C., Qing-Yi, W., and Jian-Guo, F., et al. The 3, 4-dihydroxyl groups are important for trans-resveratrol analogues to exhibit enhanced antioxidant and apoptotic activities. Anticancer Res. 2004; 24: 999-1002.
29. Howitz, K. T., Bitterman, K. J. and Cohen, H. Y., et al. Small molecule activators of sirtulins extend Saccharomyces cerevisiae lifespan. Nature 2003; 425: 191-196.
30. Baur, J. A., Pearson, K. J., and Price, N. L., et al. Resveratrol improves health and survival of mice on a high-calorie diet. Nature 2006; 444: 337-342.
31. Lio, B.L., Zhang, X., Zhang, W, Zhen H.N. New enlightenment of French paradox: Resveratrol's potential for cancer chemoprevention and anti-cancer therapy. Cancer Biol Ther. 2007; 6: 1833-1836.
32. Li, F., Gong, Q., Dong, H., Shi, J. Resveratrol, a neuroprotective supplement for Alzheimer's disease. Curr Pharm. Des. 2012; 18(1): 27-33.
33. Sharma, S., Anjaneyulu, M., Kulkarni, S. K., Chopra, K. "Resveratrol, a polyphenolic phytoalexin, attenuates diabetic nephropathy in rats," Pharmacology, vol. 2006. 76 (2), pp. 69–75.
34. Palsamy, P., and Subramanian, S. "Resveratrol protects diabetic kidney by attenuating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via Nrf2-Keap1 signaling," Biochimica et Biophysica Acta, vol. 2011; 1812(7):719–731.
35. Berbee, J.F., Wong, M.C., Wang, Y., vander Hoorn, J.W., Khedoe, P.P., van Klinken, J.B., et al. Resveratrol protects against atherosclerosis, but does not add to the antiatherogenic effects of atorvastatin, in APOE*3- Leiden. CETP mice. J Nutr Biochem. 2013; 24:1423-1430.
36. George, J., Singh, M., Srivastava, A.K., et al. Resveratrol and black tea polyphenol combination synergistically suppress mouse skin tumors growth by inhibition of activated MAPKs and p53, PLoS ONE. 2011; 6: e23395.
37. Chen, Tzu-Ying. Anti-Obesity Effects of Resveratrol, Black Tea Extract, and Caffeine in Mice. 2008.
38. Bergmeyer, H.U. Method of enzymatic analysis, second ed. Vevlag chemie, weinhein 1965; Academic Press, London.
39. Young, D.S., Clin Chem.1975; 21.10
40. Koracevic, D., Koracevic, G., Djordjevic V., Andrejevic S., Cosic, V. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001; 54: 356-361.
41. Ellman, G.D., Tissue sulphydryl groups. Arch. Biochem. Biophys. 1959; 82-70.
42. Ohkawa, H., and Ohishi, N. Assay form lipid peroxides in animal tissue by thiobarbituric acid reaction. Analytical Biochem.1979; 95: 359-364.

Source of support: Nil, Conflict of interest: None Declared