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Promising Potential: The protective Effects of a Green Tea and Resveratrol Combination on Heart Function in Rodents Enduring Isoproterenol-Induced Myocardial Infarction

Sonakshi Antal[1], Achal Anand[2], Alok P Singh[3], Jayendra Kumar[4], Rohit Pandey [5]

[1]Phd (Pharmacology) SRM Modinagar College of Pharmacy, SRMIST Delhi NCR Campus, Modinagar, Ghaziabad 20120
[2]M. Pharm (Pharmaceutics) SRM Modinagar College of Pharmacy, SRMIST Delhi NCR Campus, Modinagar, Ghaziabad 201204
[3]Phd (Pharmaceutics) SRM Modinagar College of Pharmacy, SRMIST Delhi NCR Campus, Modinagar, Ghaziabad 201204
[4]Phd (Pharm. Chemistry) SRM Modinagar College of Pharmacy, SRMIST Delhi NCR Campus, Modinagar, Ghaziabad 201204
[5]M. Pharm (Pharmaceutical Chemistry) Dr. KN Modi Institute of Pharmaceutical Education and Research, Old Cloth Mill, Compound, Opp SBI Main Branch, Modinagar, District Ghaziabad, UP Pin 201204

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KEYWORDS
Antioxidant, Green Tea, Myocardial Infarction, Isoproterenol, Resveratrol.

ABSTRACT:
When administered in large amounts, isoproterenol— a synthetic, non-selective adrenoceptor agonist— can cause severe oxidative stress and infarct-like necrosis in the myocardium. There are two types of resveratrol, a naturally occurring polyphenol: trans and cis. The cardioprotective, neurological, antidepressant, and antioxidant properties of resveratrol have been extensively studied in clinical investigations. Green tea is one of the three types of tea produced from the Camellia sinensis plant, with other varieties including oolong tea and black tea. The current study demonstrates that administering isoproterenol (ISO) to Wistar albino rats at a dosage of 85 mg/kg leads to significant cardiotoxicity. However, after a 30-day pretreatment with resveratrol-rich green tea extract, we observed cardiac protection. This treatment reversed the harmful effects indicated by levels of LDH, AST, CK-MB, and TBARS, while also increasing myocardial endogenous antioxidants such as GSH, TAC, and CAT.

1. Introduction

In the 20th century, populations all around the world experienced significant changes in lifestyle. Many advancements in science and technology that now have an impact on every aspect of human existence have contributed to these shifts. Fast food and sedentary lifestyles have replaced agrarian diets and active lifestyles in the majority of human societies.[1] These changes have fueled the epidemic of obesity, diabetes, hypertension, dyslipidemia, and cardiovascular illnesses in conjunction with rising cigarette use (CVDs). Across the entire world, Heart and blood vessel illnesses (CVDs) kill most people. About 17.5 million deaths around the world in 2012 were thought to be caused by CVDs, or 31% of all fatalities. [2] By 2020, Stroke and cardiovascular disease will pass cancer as the world's leading causes of death and disability, predicts the World Health Organization (WHO). [3] A heart attack is another name is myocardial infarction (MI). MI happens when the blood supply to a portion of the heart is cut off, which causes ischemia. It can result in infarction, which results in the death of cardiac muscle tissue, if untreated for a long enough time. [4] Despite fast breakthroughs in the treatment of coronary artery disorders, MI continues to be the leading cause of death worldwide and is a significant pathological problem. Coronary atherosclerosis causes more than 80% of AMI case.[5-6]

Isoproterenol
It is a synthetic, non-selective -adrenoceptor agonist that, in myocardial, causes infarct-like necrosis and severe oxidative stress at high dosages. [1,6] Moreover, it is known to produce free radicals and promote lipid peroxidation, both of which harm the cardiac membrane irreparably.[7]One of the major contributing reasons to MI is when isoproterenol breaks down on its own, creating these very harmful free radicals. [8] The heart damage caused by isoproterenol in rats paradigm is a widely used standard model for assessing the cardioprotective capabilities of different products. The

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myocardial changes caused by isoproterenol resemble those that happen after a MI in people in certain ways. [9-11]

Resveratrol

Resveratrol, an inherent polyphenolic compound, is present in several food sources such as grapes and mulberries, utilized as a remedy. There are two types of resveratrol (trans and cis). Trans-resveratrol is a highly absorbable form, but because of its quick metabolism and excretion, it has a relatively poor bioavailability. [12-13] The substance is thought to have strong antioxidant properties that assist cellular health. The physiological effects of resveratrol have been examined in numerous clinical trials and have included anticancer, cardioprotective, neuroprotective, depressive, and antioxidant activity. [14-16]

Green tea (*Camellia sinesis*).

Camellia sinensis is of three types including green. Nlack, and oolong teas are available. Green tea's health advantages are attributed to catechins, a polyphenol that makes up 30% of dry leaves weight. [1, 15,18] The physiological effects of catechins include antioxidant, cardioprotective, anti-inflammatory, anti-diabetic, and antibacterial properties. [1-3.18]

2. Materials and Methods

Drugs

The supplier of resveratrol was Zenith Nutrition's Pvt. Ltd. in Bangalore, India. We bought green tea extract from Sanat Product Ltd. in New Delhi, India. The supplier of isoproterenol was Sigma-Aldrich in India and commercial diagnostic kits were obtained from Agappe Pvt. Ltd., Kerala, India. All other reagents were of analytical grade. [16,20-22]

Animals Studies

For this investigation, 150–200 g Wistar albino rats were used in the study after obtaining the approval of the Institute's Animal Ethics Committee (Approval code no**. 386/PO/ReBi/SL/01/CPCSEA**). Animals were fed on a standard pellet diet and water ad libitum and maintained at 24–28°C temperature and relative humidity (30% - 70%). Animals marked as fasted were deprived of food for 16 hours, but had free access to water. [19, 22] Green tea extract and resveratrol were given orally to the animals. They were maintained in the typical laboratory environment and given unlimited access to the commercial diet.

Experimental Protocol

Groups (n=5)	Drug Treatment, dosage & route of administration
Control	Normal saline (1 ml) orally for 1month + normal saline (0.1 ml) *sc* on 29th & 30th day.
Isoproterenol (ISO)	Normal saline (1 ml) orally daily for 30 days + ISO (85 mg/kg) *sc* on 29th & 30th day [8].
Resveratrol + ISO	Resveratrol (20 mg/kg) orally daily for 30 days + ISO (85 mg/kg) *sc* on 29th & 30th day [22].
Green tea extract + ISO	Green tea extract (400 mg/kg) orally daily for 30 days + ISO (85mg/kg) *sc* on 29th & 30th day [23].
Resveratrol + Green tea extract *Per se*	Resveratrol (20 mg/kg) + Green tea extract (400 mg/kg) orally daily for 30 days.
Resveratrol + Green tea extract + ISO	Resveratrol (20 mg/kg) + Green tea extract (400 mg/kg) orally daily for 30 days + ISO (85 mg/kg) *sc* on 29th & 30th day.

* SC= subcutaneous

General Observations

During the treatment period, water consumption, food consumption and mortality rates were noted. [20]

Blood Sample Collection and Analysis

At the end of treatment blood was collected from retro orbital plexus by anesthetizing the rats with thiopental

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sodium (35mg/kg body weight, intra peritoneal) and serum was separated by centrifugation at 2000rpm. [17]

Biochemical Estimation in Serum

Serum was used to analyze various biochemical parameters such as determinations of cardiac biomarkers lactate dehydrogenize (LDH), and creatinine kinase MB (CK-MB) by using commercial diagnostic kits (Agappe Pvt. Ltd., Kerala, India).

Biochemical Estimation in Tissue

Determination of tissue antioxidants At the end of the experimentation hearts were excised from rats and homogenate in 0.1M Tris buffer (pH7.4) and the separated homogenates were used for estimation of heart antioxidants like super oxide dismutase (SOD) , Reduced glutathione (GSH), Catalase [and Thiobarbituric acid reactive substances (TBA) [23-27]

Histopathological Studies of Heart

After removal of myocardial tissue immediately washed with ice cold saline to remove all the blood and fixed in 10% buffered neutral formalin solution. After fixation was complete, tissues were embedded in paraffin and serial sections were cut in to 0.5µm. Each section was stained with haematoxylin and eosin. The sections were examined under light microscope and histograms were taken. [28-30]

Statistical Analysis

Results are expressed as mean ± SE. Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by the Tukey–Karmer multiple comparison tests. $P < 0.05$ was considered significant. [31]

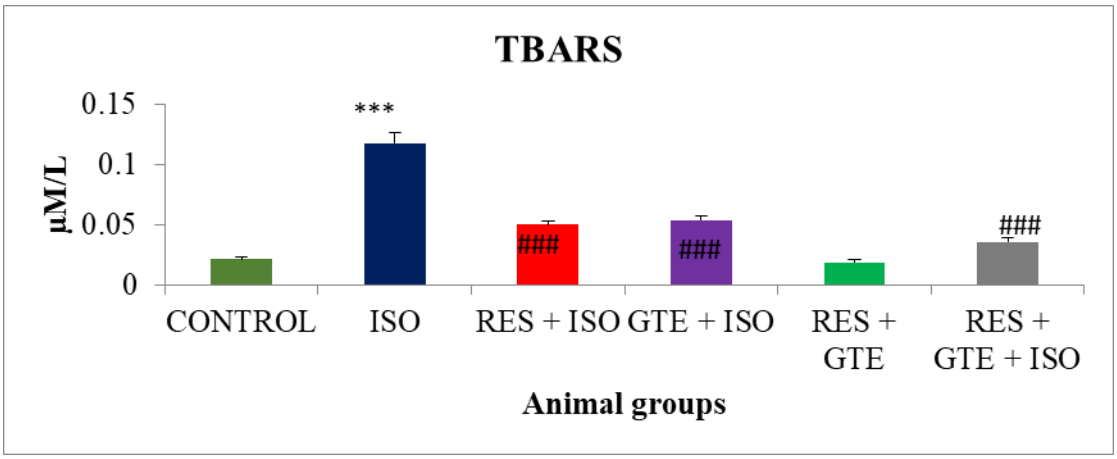
3. Results and Observations

Heart-to-body Weight Ratio.

Groups	HW/BW ratio
CONTROL	3.466 ± 0.094
ISO	4.828 ± 0.164***
RES + ISO	3.956 ± 0.195#
GTE+ ISO	4.647 ± 0.229
RES+ GTE + ISO	3.608 ± 0.063###
RES + GTE	3.578 ± 0.179

Heart weight / body weight (HW/BW) ratio in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol. Results are shown as mean ± SEM (n = 5) with *** p < 0.001 (CONTROL vs ISO), ###P < 0.05 (RES + ISO vs ISO), and P < 0.001 (RES + GTE + ISO vs ISO) using one-way ANOVA with Tukey-Kramer test. [31]

Bar graph showing results of TBARS in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol. Values are mean ± SEM (n = 5) with *** P < 0.001 (control versus ISO) and ###P < 0.001 (RES + ISO, GTE + ISO, RES + GTE + SOs ISO using one-way). [24]



Bar graph showing results of GSH in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol. Results are shown as mean ± SEM (n = 5), with *** P < 0.001 (CONTROL vs ISO), ### P < 0.001 (RES + ISO vs ISO, RES +GTE + ISO vs ISO), and # P < 0.05 (GTE + ISO) using one-way ANOVA with Tukey-Kramer comparison test. [33]

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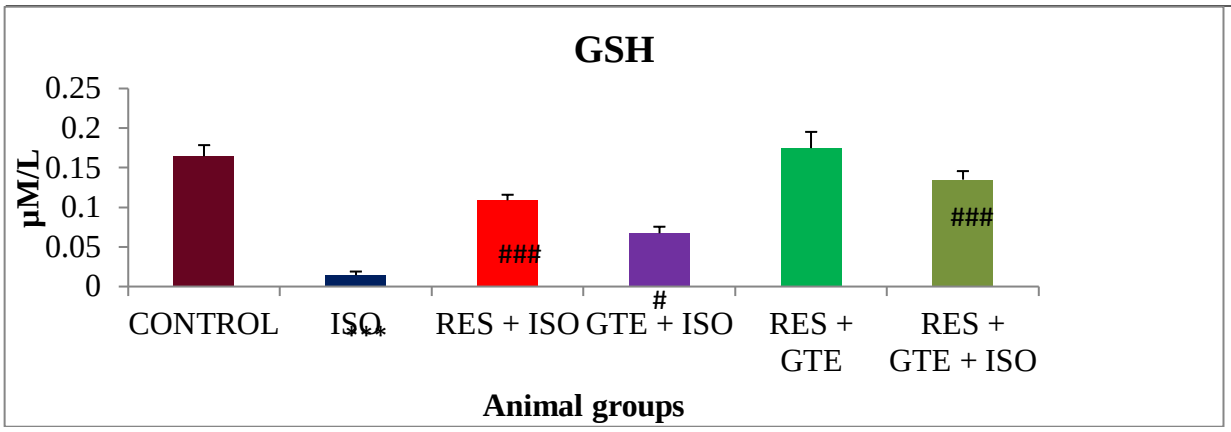
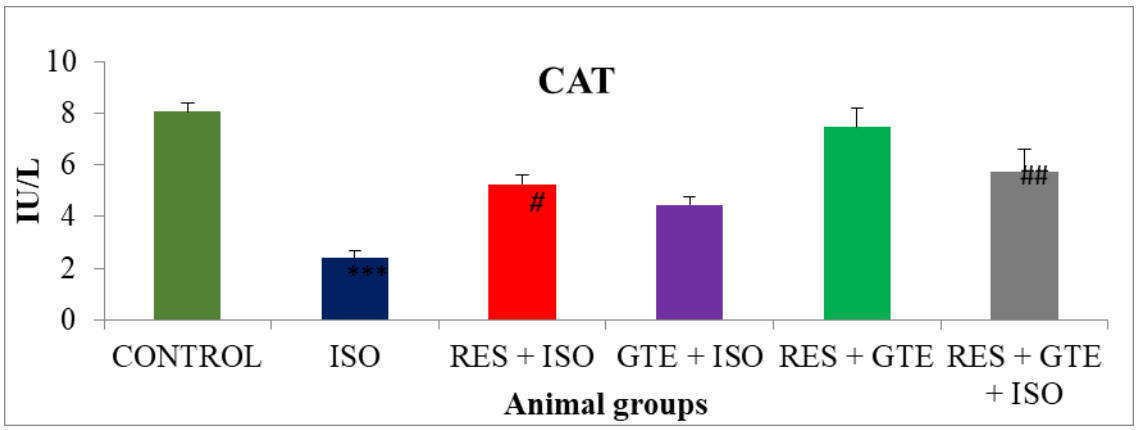


Bar graph showing results of Catalase in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol. Mean ± SEM (n = 5) with *** P < 0.001 (CONTROL vs ISO), #P < 0.001 (RES + ISO vs ISO), and ##p < 0.001 (RES + GTE + SO vs ISO) using one-way ANOVA with Tukey-Kramer test. [28]



Biochemical Estimations in Serum

Groups	LDH (IU/L)	AST (IU/L)	CK-MB (IU/L)	TAC (µM/L)
CONTROL	1154.16 ± 72.75	8.205 ± 3.24	142.36 ± 7.794	3.028 ± 0.243
ISO	2526.328 ± 131.07***	51.534 ± 5.22***	477.045 ± 33.31***	0.953 ± 0.04554***
RES + ISO	1763.3 ± 106.33##	27.697 ± 4.22#	223.606 ± 13.74###	2.025 ± 0.3009#
GTE + ISO	1869.098 ± 185.74#	25.264 ± 7.79##	349.286 ± 11.74###	1.78 ± 0.2322
RES + GTE	1436.288 ± 147.32	21.016 ± 3.335	224.537 ± 11.187	2.420 ± 0.3172
RES + GTE + ISO	1644.678 ± 101.84###	8.529 ± 2.28##	196.556 ± 17.117###	2.243 ± 0.1577##

Bar graph showing results of AST in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol +

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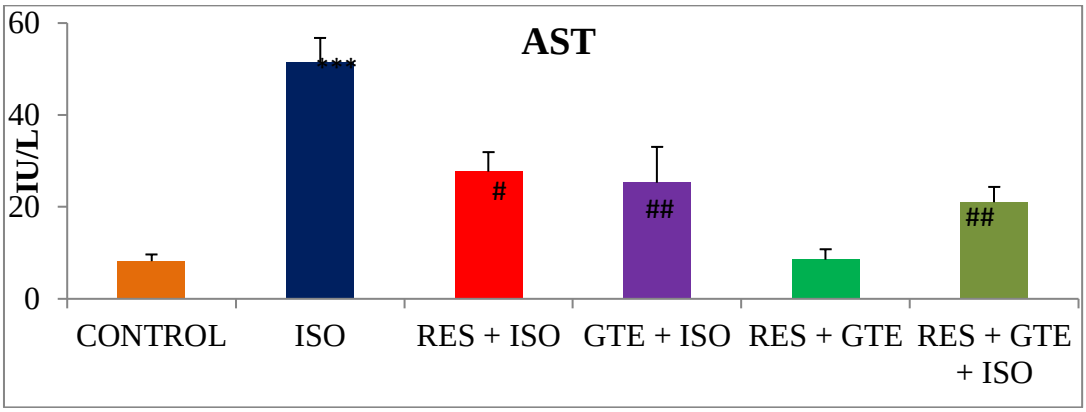
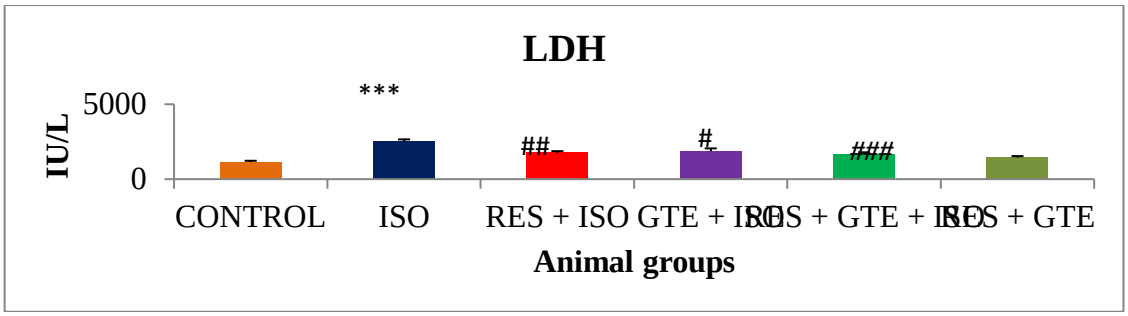
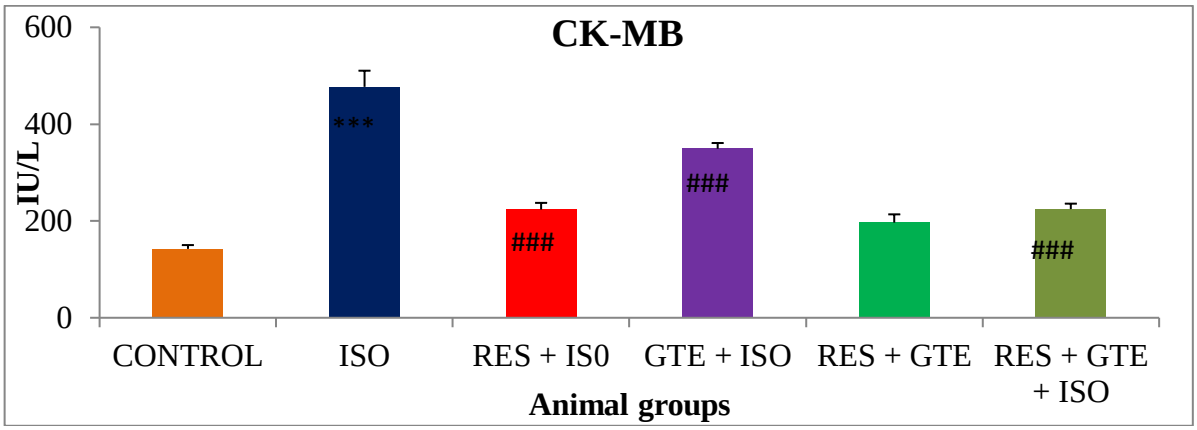
isoproterenol. Results are shown as mean ± SEM (n = 5) with *** p < 0.001 (CONTROL versus ISO), # P < 0.05 (RES + ISO vs ISO), ##p < 0.01 (GTE + ISO vs ISO), and ##p < 0.01 (RES + GTE + ISO vs ISO) using one-way ANOVA with Tukey-Kramer comparison test. [31]



Bar graph showing results of LDH in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol. Results are presented as mean ± SEM (n = 5), with *** P < 0.001 (control vs ISO), ##p < 0.01 (RES + ISO vs ISO), #P <0.05 (GTE + ISO vs ISO), and ### P < 0.001 (RES + GTE + ISO vs ISO). With one-way ANOVA and Tukey-Kramer comparison test.



Bar graph showing results of CK-MB in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol. Results are shown as mean ± SEM (n = 5), with *** p < 0.001 (control vs ISO) and ###p < 0.001 (RES + ISO, GTE + ISO, and RES + GTE + ISO versus ISO) using one-way ANOVA with Tukey-Kramer comparison test. [32]



Bar graph showing results of TAC in various groups. Control, ISO, isoproterenol; RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol. Results are presented as mean ± SEM (n = 5), with *** p < 0.001 (CONTROL versus ISO), # P < 0.05

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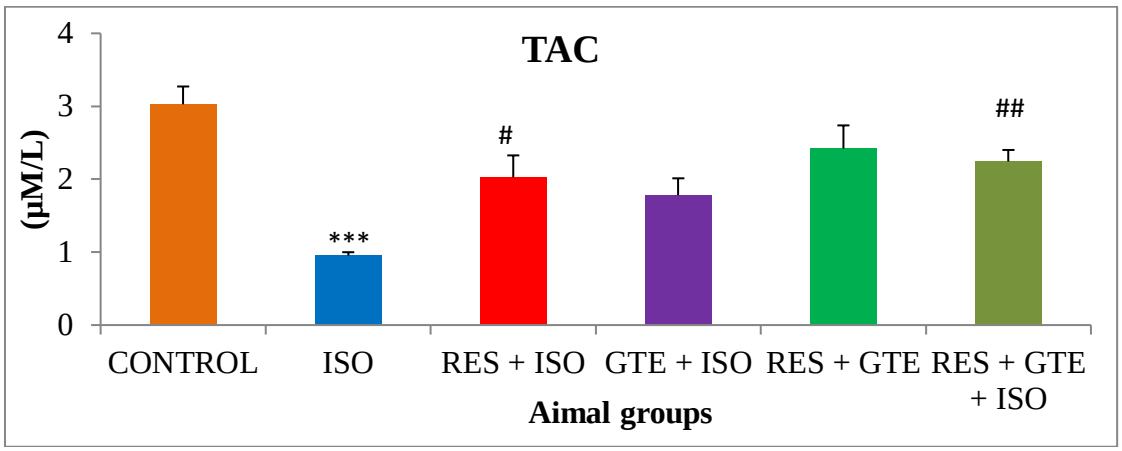
(RES + ISO vs ISO), and ##P < 0.01 (RES + GTE + ISO vs ISO) using one-way ANOVA with Tukey-Kramer test.[29,32]



Histopathological studies

Sham-operated rats had normal myocardial cell nuclei and no broken myocardial fibers. A greater degree of inflammatory cell infiltration and broken myocardial fibers were observed in control rats. In the RES + ISO, resveratrol + isoproterenol; GTE + ISO, green tea extract + isoproterenol; RES + GTE+ ISO, green tea extract + resveratrol + isoproterenol and positive control groups, the degrees of inflammatory cell infiltration and numbers of broken myocardial fibres remained within the normal ranges. [31]

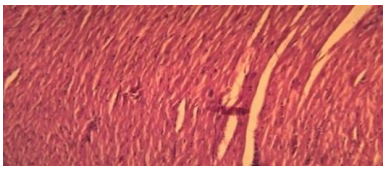


Fig. 1 Normal control group showing normal architecture of myocardial with no infiltration and vacuolation of cells

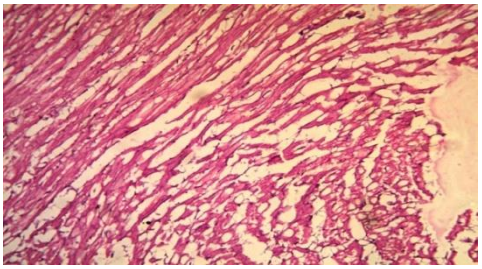


Fig. 2 (ISO) isoproterenol group showing remarkable disintegration of myocardium muscle fibers, vacoulation and the presence of pyknotic nucleus.

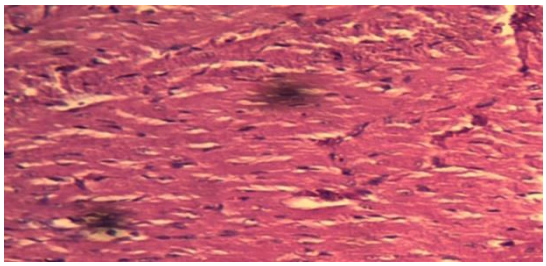


Fig.3 (RES + ISO) Resveratrol treated groups showing almost normal architecture of myocardial fibers.

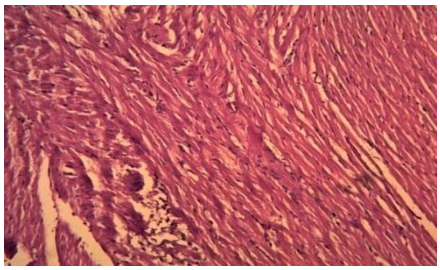


Fig. 4 (GTE + ISO) green tea extract treated groups shows few pyknotic nucleus and vacuoles

4. Summary and Discussion

The current study showed that administering ISO (85 mg/kg) to Wistar albino rats resulted in severe cardiotoxicity, as evidenced by elevated cardiac TBARS, elevated serum marker enzymes (LDH, AST, and CK-MB), and decreased myocardial endogenous antioxidants and enzymes (GSH, TAC & CAT). It was also seen in the heart tissue through histopathology, showing disorganized myocardial fibers, cytoplasmic vacuolation, and pyknotic nuclei.[28] Moreover, ISO-treated groups showed an increase in the heart weight to body weight ratio. Calculating infarct size also showed that animals who had received ISO treatment had an elevated

infarct area (64.47%). Pretreatment for 30 days with green tea extract (400 mg/kg) and resveratrol (20 mg/kg) reversed the increase in LDH, AST, CK-MB, and TBARS, demonstrated cardiac protection, and restored levels of myocardial endogenous antioxidants (GSH, TAC, and CAT). [29]

Moreover, it reduced vacuolation and preserved the myofibrils' integrity as seen under a light microscope. Furthermore, using these medications resulted in a reduction in infarct size compared to the ISO group. Before treatment with a 20 mg/kg mix of resveratrol and green tea extract for 30 days, the heart was better protected because the levels of TBARS in the heart tissue and the marker enzymes LDH, AST, and CK-MB in the blood were returned to normal. [1,8,27], [32] The myocardium also experienced a dramatic restoration of GSH, TAC, and CAT levels. The per se groups showed no cardiac toxicity, and the biochemical markers and enzymes remained normal. Histopathological analyses also revealed no ,myocardial disruption. We conclude that all of the data related to green tea extract, resveratrol, and their combination demonstrated their cardio-preventive impact. However, the combination of resveratrol and green tea extract shows more excellent protection than resveratrol and green tea extract alone. [3,19,26] However, the biochemical, histological, and infarct size observations among the resveratrol and green tea extract-treated groups suggested that resveratrol was more effective in protecting against ISO-induced heart injury. The rats' heart weight to body weight ratio increased considerably after receiving isoproterenol therapy. The cardiac weight-to-body weight ratio significantly decreased in the resveratrol-treated group, while it did not differ substantially in the green tea group. However, the combination group revealed a considerable variation in this ratio. Also, combining resveratrol and green tea extract is preferable to taking them separately for a large population suffering from heart disease and other lifestyle-related illnesses. Both medications are herbal nutritional supplements that are readily available, affordable, and offer a variety of health advantages. [13, 20, 25,]

5. Conclusion

Our studies have demonstrated the protective effects of green tea extract and resveratrol against isoproterenol-induced myocardial infarction. While green tea extract does not provide the same degree of cardiac protection as resveratrol, their combined effect is more effective than either treatment alone.

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