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EVALUATION OF CARDIOPROTECTIVE EFFECTS OF CAMELLIA SINENSIS ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION

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Keywords:

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ABSTRACT:

Background: Black tea is rich in polyphenols which are antioxidants. The most abundant polyphenolic compounds are flavonols and catechins. Black tea antioxidants showed protective actions against chronic diseases like cardiovascular diseases due to their antioxidant action.

Objective: The present study was conducted to evaluate the cardioprotective effects of *Camellia sinensis* on isoproterenol induced myocardial infarction in Wistar albino rats. Myocardial infarction (MI) is a condition in which a part of the heart muscle suddenly loses its blood supply, so the myocardium gets destructed and becomes ischemic. *Camellia sinensis* is a species of plant from which leaves and leaf buds are taken to produce tea. Myocardial infarction was induced by subcutaneous administration of Isoproterenol. Isoproterenol shows positive inotropic and chronotropic effects on heart. Oxidative products of drug cause cytotoxicity and myocardial damage.

Methods: Wistar albino rats were divided into 8 groups, each group containing 8 rats. The following treatment was given for 21 days. Group 1: NS 1mg/kg (orally). Group 2: NS+ISO (85 mg/kg, s.c.). Group 3: BTE 1 (4mg/kg, orally) +ISO. Group 4: BTE2 (8mg/kg) +ISO. Group 5: BTE3 (12mg/kg) +ISO. Group 6: BTE3 per se. Group 7: STD (10mg/kg) +ISO. Group 8: BTE3+STD+ISO. On 20th and 21st day, ISO (85mg/kg) was given. After 24 hrs of last treatment, blood was collected, soon after animals were sacrificed and heart tissues were collected for biochemical and histopathological evaluations.

Result: Administration of BTE reduced the levels of GSH, TBARs, SOD, Catalase, LDH and SGOT. Standard drug treatment alone and in combination with BTE3 protected the heart by reduction in the infarct size.

Conclusion: Isoproterenol induced myocardial infarction in rat heart, which was significantly prevented by *Camellia sinensis* and carvedilol treatment alone and in combination.

INTRODUCTION: Myocardial infarction (MI) is a condition in which a part of the heart muscle suddenly loses its blood supply, so the myocardium gets destructed and becomes ischemic [12, 23, 30]. The most common cause of MI is the formation of blood clot (thrombosis) inside a coronary artery, or its branches. So the blood flow to a part of the heart gets blocked. Other conditions which block a coronary artery are: inflammation of the coronary arteries (rare); a stab wound to the heart; a blood clot forming elsewhere in

the body (for example, in a heart chamber) and travelling to a coronary artery where it gets stuck; taking cocaine which can cause a coronary artery to go into spasm. Apoptosis also plays a role in the process of tissue damage subsequent to myocardial infarction. The most common symptom of an MI is severe chest pain. The pain may travel up into the jaw and down to left arm or down both arms. The patient sweat, feels sick, feel faint and feel short of breath. Different types of MI are classified according to heart ECG (heart tracing). They are ST elevation MI (STEMI) and non-ST elevation MI (NSTEMI) and the treatment depends upon the type of MI [30]. MI needs immediate medical emergency.

The following drugs can be used: Antiplatelet, Heparin, Beta blockers, Pain killer, Oxygen, Nitroglycerin. Cardiac rehabilitation can optimize heart function. It includes Physical exercise which can normalize cholesterol levels, blood pressure, weight, stress and mood. Risk factors for MI include smoking, hypercholesterolemia, hyperlipoproteinemia, diabetes, hypertension, and obesity. Free radicals are species of atoms containing an odd (unpaired) number of electrons [15, 18]. Free radicals can rapidly react with other molecules and starting a chain reaction of free radical formation. They miss an electron, so steal it from another molecule to become saturated. But now, a victim molecule has become a free radical and precedes a chain reaction [14]. Antioxidants are species of molecules capable of interacting with free radicals and terminating the chain reactions, so preventing the cellular damage. Antioxidants give up their own electrons to free radicals. So, they become a free radical which is not harmful. After free radical gained the electron from an antioxidant, its electrons will become completed and it no longer attacks the cell [10].

Animal models data suggest a correlation between free radical injury and promotion of myocardial decompensation and heart failure [34]. *Camellia sinensis* is the plant species from which leaves and leaf buds are used to produce tea [27]. The most abundant forms of polyphenolic antioxidants in black tea are flavonols and Catechins which prevent the formation of free radicals [5]. Black tea flavonoids can reverse endothelial dysfunction [39] and reduce superoxide radical and alkyl peroxy radicals. They also increase endothelium-dependent dilatation [16]. Present investigation was planned to evaluate the effect of ethanolic extract of ***Camellia sinensis*** on Isopproterenol induced myocardial infarction in rats.

MATERIALS AND METHODS: Wistar albino rats were divided into 8 groups, each group containing 8 rats. The following treatment was given for 21 days.

TABLE 1 : DOSAGE AND TREATMENT

GROUP	TREATMENT	NO. OF ANIMALS	DOSE / ROUTE
1	NS	08	1 mg/kg orally
2	NS+ISO	08	1mg/kg(orally)+85mg/kg(sc)
3	BTE1+ISO	08	4mg/kg(orally)+85mg/kg(sc)
4	BTE2+ISO	08	8mg/kg(orally)+85mg/kg(sc)
5	BTE3+ISO	08	12mg/kg(orally)+85mg/kg(sc)
6	BTE3 PER SE	08	12 mg/kg (orally)
7	STD+ISO	08	10mg/kg(orally)+85mg/kg(sc)
8	BTE3+STD+ISO	08	12mg/kg(orally)+10mg/kg(orally)+85mg/kg(sc)

NS: Normal saline, ISO: Isoproterenol, BTE: Black tea extract, STD: Standard

On 20th and 21st day, ISO (85 mg/kg) was given which induces myocardial infarction. After 24 hours of the last treatment, blood was collected from tail vein under light ether anaesthesia, soon after animals were sacrificed and heart tissues were collected and washed in ice-cold saline and a homogenate was prepared in 0.1 M Tris HCL buffer. Homogenate was centrifuged and supernatant was used for biochemical assay [35].

Black tea was allowed to get dried at room temperature and was extracted by ethanol by soxhlet extractor for 12 hours. Then the extract was allowed to evaporate to dryness under reduced pressure and controlled temperature in rotary evaporator. The product which is an ethanolic extract of black tea is dark brown in color and semi-solid in nature. It was dispersed in 0.5% CMC suspension and administered by oral route (4mg/kg, 8mg/kg, and 12mg/kg).

The following parameters were estimated according to the reported procedures:

Biochemical estimation (in cardiac tissue)

1. Thiobarbituric Acid Reactive Substance [32]
2. Glutathione Peroxidase [37]
3. Superoxide Dismutase [29]
4. Catalase [8]
5. Protein [25]

Biochemical estimation (in serum)

1. Lactate Dehydrogenase [46]
2. Aspartate Aminotransferase [47]

Histopathological Studies: To carry out histopathological examination the hearts were excised and immediately fixed in 10% buffered formalin. The ventricular mass was sectioned from the apex to the base of the heart, which was embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Five-micrometer thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken.

Statistical Evaluations: The results were presented as ± SEM. One way ANOVA was used to access the significance of difference between groups. $p< 0.05$ was considered significant [35].

RESULT AND DISCUSSION: Myocardial infarction is the leading cause of death in developed countries [2]. An MI requires immediate medical attention as it is a medical emergency. The commonest cause of an MI is the formation of blood clot (thrombosis) inside a coronary artery, or its branches. The possible beneficial effects of tea consumption in the prevention of cancer and cardiovascular diseases have been demonstrated in animal models. Tea leaves are rich in flavonols and catechins which are potent antioxidants. They scavenge cell damaging free radicals and detoxify them [5]. Black tea antioxidants inhibit oxidation of LDL & VLDL, so inhibit atherogenesis.

Catechins in green and black tea are powerful antioxidants, capable of rapid reduction of superoxide radical and alkyl peroxy radicals. Reactive oxygen species (ROS) are known mediators of intracellular signal cascades. Excessive production of ROS may lead to oxidative stress, loss of cell function, and cell death by apoptosis or necrosis [7, 11, 40]. Oxidative products of isoproterenol especially oxygen/ or oxygen derived free radicals are responsible for the cardiotoxicity [31].

Isoproterenol produces cardiomyocyte necrosis and fibrosis. Isoproterenol infused rats show cardiac remodeling with severe myocardial hypertrophy and myocardial injury, which are resulted from rise in cardiac generation of ROS [3, 7, 9, 11, 21, 38, 40, 43]. Disruptions of contractile proteins were seen in hearts failing due to oxidized isoproterenol. Carvedilol is a non-selective beta blocker/alpha-1 blocker and used in the treatment of mild to moderate congestive heart failure (CHF). Carvedilol inhibits endothelial cell apoptosis and show beneficial effects in the management of heart failure [36]. The use of carvedilol reduces morbidity and mortality rates in CHF patients [33]. In our study, the carvedilol was used as standard drug.

Lipid peroxide is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflect the various stages of the disease and its complications. Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction viz., CPK, LDH and transaminases (SGOT, SGPT) and once metabolically damaged, releases its content into the extra cellular fluid (ECF) [35].

Thiobarbituric Acid Reactive Substances (TBARS) assay is used for screening and monitoring lipid peroxidation, a major indicator of oxidative stress [1, 24, 41]. The assay provides important information about free radical activity in disease states. Malondialdehyde (MDA) is one of the lipid peroxidation products which are measured in this assay.

Glutathione peroxidase (GPx) plays a protective role in oxidative stress-induced apoptosis. Cells which are defective in GPx were more easily brought to apoptotic cell death by peroxides [19]. Glutathione peroxidase assay is done to determine the extent of oxidative stress in the cell which can be rised due to diseased state.

Catalase catalyze the decomposition of hydrogen peroxide to water and oxygen [6] and facilitate the removal of hydrogen peroxide. Catalase assay is done to estimate the oxidative state of the cell.

Superoxide Dismutase (SOD) catalyzes the dismutation of the superoxide anion (O2-) into hydrogen peroxide and molecular oxygen [28]. It's over expression protects against apoptosis and promote cell differentiation [44]. Estimation of SOD gives information regarding the cell oxidative stress.

Protein assays are used to determine protein concentration. Protein estimation of protein concentration is necessary in protein purification, electrophoresis, cell biology, molecular biology, and other research applications.

Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate along with interconversion of NADH and NAD^+. The enzyme LDH is contained within the tissues and cells, and is released into the bloodstream when cells are damaged or destroyed. So the LDH test is used as a general marker of injury to cells.

AST is defined as a biochemical marker in the diagnosis of acute myocardial infarction (MI) [13]. The enzyme is released into the serum in case of tissue injury, so it may increase as a result of myocardial infarction and liver damage.

TBARS level was increased in the group receiving Isoproterenol, while, GSH, LDH, SGOT, SOD and Catalase levels were reduced in the group receiving Isoproterenol. TBARS level was reduced while GSH, LDH, SGOT, SOD and Catalase levels were increased after administration of black tea extract and carvedilol alone and in combination.

Isoproterenol induces myocardial infarction in rat heart, which was significantly prevented by *Camellia Sinensis* and carvedilol treatment alone and in combination.

Biochemical result was supplemented by histo-pathological evaluation.

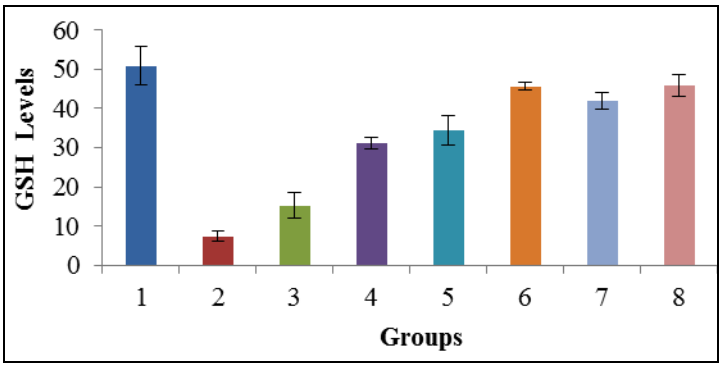

FIGURE 1: EFFECT OF BTE ON GSH LEVELS IN ISOPROTERENOL INDUCED CARDIOTOXICITY IN RATS

Effect of black tea on level of GSH:

- Tissue GSH level was increased to a very significant level (P<0.01) in the Isoproterenol induced myocardial infarction group (group II) as compared to the normal control group (group I).
- Elevated tissue GSH level was decreased to a non significant (P> 0.05), significant (P<0.05) and highly significant (P<0.01) level with BTE in doses 4, 8 and 12 mg/kg respectively in Isoproterenol induced myocardial infarction rats.
- Elevated tissue GSH level was decreased to a highly significant level (P<0.01) with carvedilol (10 mg/kg) (group VII) as well as in a combination of carvedilol (10 mg/kg) and BTE_3 (12 mg/kg) (group VIII).

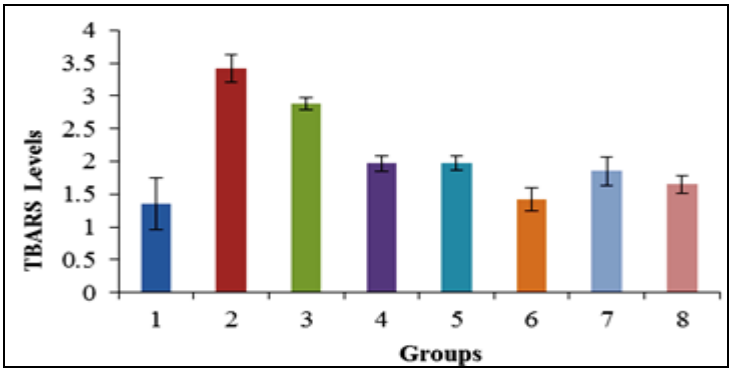

FIGURE 2: EFFECT OF BTE ON TBARS IN ISOPROTERENOL INDUCED CARDIOTOXICITY IN RATS

Effect of Black Tea on level of TBARs:

- Tissue TBAR level was increased to a very significant level (P<0.01) in the Isoproterenol induced myocardial infarction group (group II) as compared to the normal control group (group I). The increased levels of thiobarbituric acid reactive substances (TBARS) indicate the excessive formation of free radicals and activation of lipid

peroxidation system resulting in irreversible damage to the heart in animals subjected to ISPH stress.

- Elevated tissue TBAR level was decreased to a non significant (P> 0.05), significant (P<0.05) and highly significant level (P<0.01) with BTE in doses 4, 8 and 12 mg/kg respectively in Isoproterenol induced myocardial infarction rats.
- Elevated tissue TBAR level was decreased to a highly significant level (P<0.01) with carvedilol (10 mg/kg) (group VII) as well as in a combination of carvedilol (10 mg/kg) and BTE_3 (12 mg/kg) (group VIII).

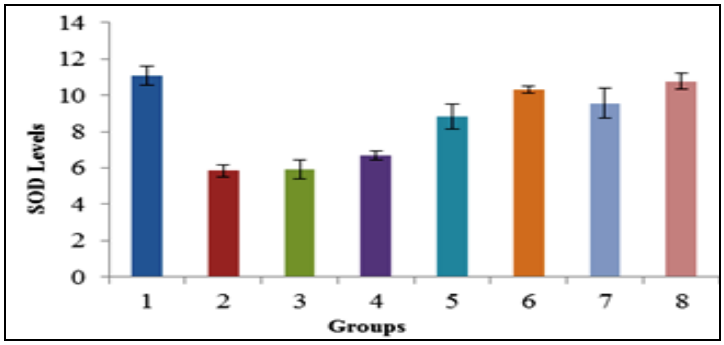

FIGURE 3: EFFECT OF BTE ON SOD LEVELS IN ISOPROTERENOL INDUCED CARDIOTOXICITY IN RATS

Effect of Black Tea on level of SOD:

- Tissue SOD level was increased to a very significant level (P<0.01) in the Isoproterenol induced myocardial infarction group (group II) as compared to the normal control group (group I).
- Elevated tissue SOD level was decreased to a non significant (P> 0.05), significant (P<0.05) and highly significant level (P<0.01) with BTE in doses 4, 8 and 12 mg/kg respectively in Isoproterenol induced myocardial infarction rats.
- Elevated tissue SOD level was decreased to a highly significant level (P<0.01) with carvedilol (10 mg/kg) (group VII) as well as in a combination of carvedilol (10 mg/kg) and BTE_3 (12 mg/kg) (group VIII).

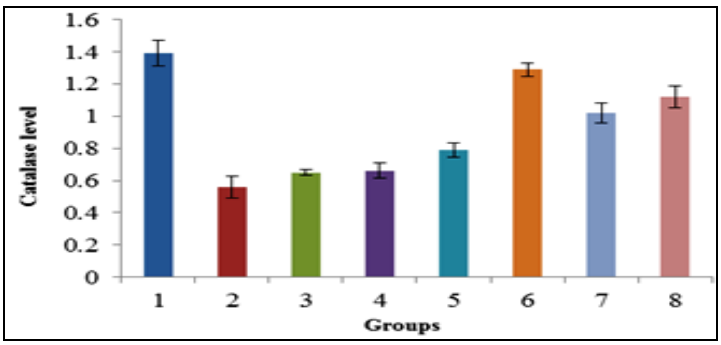

FIGURE 4: EFFECT OF BTE ON CATALASE LEVELS IN ISOPROTERENOL INDUCED CARDIOTOXICITY IN RATS

Effect of Black Tea on level of Catalase:

- Tissue catalase level was increased to a very significant level (P<0.01) in the Isoproterenol induced myocardial infarction group (group II) as compared to the normal control group (group I).
- Elevated tissue catalase level was decreased to a non significant (P> 0.05), significant (P<0.05) and highly significant level (P<0.01) with BTE in doses 4, 8 and 12 mg/kg respectively in Isoproterenol induced myocardial infarction rats.
- Elevated tissue catalase level was decreased to a highly significant level (P<0.01) with carvedilol (10 mg/kg) (group VII) as well as in a combination of carvedilol (10 mg/kg) and BTE_3 (12 mg/kg) (group VIII).

TABLE 2: THE EFFECT OF BLACK TEA EXTRACT AND CARVEDILOL ALONE AND IN COMBINATION ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

Group	Drug treatment	Dose	GSH (µg/mg protein)	TBARs (nmoles MDA/ mg protein)	SOD (units/mg protein)
I	NS	1 ml/kg	50.90 ± 4.82	1.35 ± 0.39	11.06 ± 0.52
II	NS + Iso	1 ml/kg + 85 mg/kg	7.39 ± 1.42 ***	3.42 ± 0.21 ***	5.85 ± 0.33 ***
III	BTE_1 + Iso	4 mg/kg + 85 mg/kg	15.23 ± 3.21 **	2.88 ± 0.09 *	5.92 ± 0.53 *
IV	BTE_2 + Iso	8 mg/ kg + 85 mg/kg	31.14 ± 1.58 ***	1.97 ± 0.12 ***	6.67 ± 0.24 *
V	BTE_3 + Iso	12mg/kg + 85 mg/kg	34.48 ± 3.72 ***	1.79 ± 0.11 ***	8.81 ± 0.69 **
VI	BTE_3 Per se	12 mg/kg	45.63 ± 0.90 *	1.42 ± 0.17 *	10.32 ± 0.19 *
VII	STD + Iso	10 mg/kg + 85 mg/kg	41.94 ± 2.25 ***	1.85 ± 0.22 ***	9.55 ± 0.82 ***
VIII	BTE_3 + STD + Iso	12 mg/kg + 10 mg/kg + 85 mg/kg	45.83 ± 2.89 ***	1.65 ± 0.13 ***	10.76 ± 0.43 ***

*–P > 0.05, ** –P < 0.05, ***–P < 0.01; Data presented on Mean ± SEM. Significance of data was calculated by ANOVA followed by Dunnett's test. Group I was compared with group II. All the other groups were compared with group II. n = 8 p < 0.01 was considered highly significant.

TABLE 3: THE EFFECT OF BLACK TEA EXTRACT AND CARVEDILOL ALONE AND IN COMBINATION ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS.

Group	Drug treatment	Dose	Catalase (η mole of H_2O_2 consumed /min./mg protein)	LDH (IU/L)	SGOT (IU/L)
I	NS	1 ml/kg	1.39 ± 0.08	30.25 ± 1.45	588.02 ± 45.30
II	NS + Iso	1 ml/kg + 85 mg/kg	0.56 ± 0.07 ***	48.65 ± 1.10 ***	945.09 ± 15.57 ***
III	BTE_1 + Iso	4 mg/kg + 85 mg/kg	0.65 ± 0.01 *	47.23 ± 1.18 *	895.26 ± 14.99 *
IV	BTE_2 + Iso	8 mg/ kg + 85 mg/kg	0.66 ± 0.04 *	42.25 ± 1.25 **	735.26 ± 12.70 **
V	BTE_3 + Iso	12mg/kg + 85 mg/kg	0.79 ± 0.04 **	39.98 ± 0.89 **	695.32 ± 22.23 **
VI	BTE_3 Per se	12 mg/kg	1.29 ± 0.04 *	28.95 ± 1.23 *	552.21 ± 25.34 *
VII	STD + Iso	10 mg/kg + 85 mg/kg	1.02 ± 0.06 ***	35.75 ± 1.27 ***	632.25 ± 25.90 ***
VIII	BTE_3 + STD + Iso	12 mg/kg + 10 mg/kg + 85 mg/kg	1.12 ± 0.06 ***	33.69 ± 1.36 ***	610.32 ± 56.69 ***

*–P > 0.05, ** –P < 0.05, ***–P < 0.01, Data presented on Mean ± SEM. Significance of data was calculated by ANOVA followed by Dunnett's test. Group I was compared with group II. All the other groups were compared with group II. n = 8 p < 0.01 was considered highly significant.

Histopathological Studies:

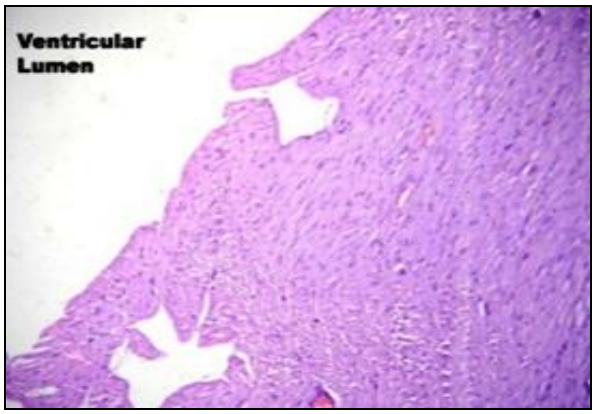

FIGURE 5: GROUP 1 NORMAL CONTROL

Photomicrograph of vehicle control group (i.e. group I) revealed cardiac fibers with normal architecture and regular morphology and showed muscle fibers with striations in cytoplasm and elongate nuclei.

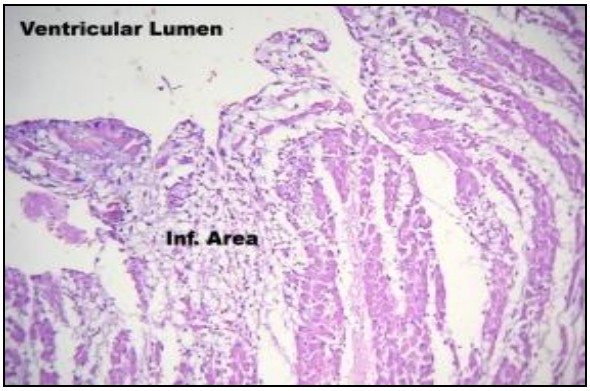

FIGURE 6: GROUP 2 TOXIC CONTROL

Photomicrograph of Toxic control group (i.e. group II) showed large areas of infarcted cardiac muscle tissue with extensive loss of muscle fibers, scattered inflammatory cells and isolated residual muscle fibers.

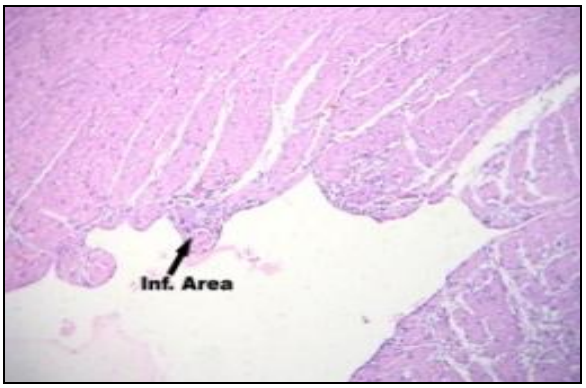

FIGURE 7: GROUP 3 BTE DOSE 1

Photomicrograph of BTE dose 1 (i.e. group III) showed small areas of infarcted cardiac muscle tissue with loss of muscle fibers, scattered inflammatory cells and isolated residual muscle fibers, thus offering no protection as compared to the Toxic control group (i.e. group II).

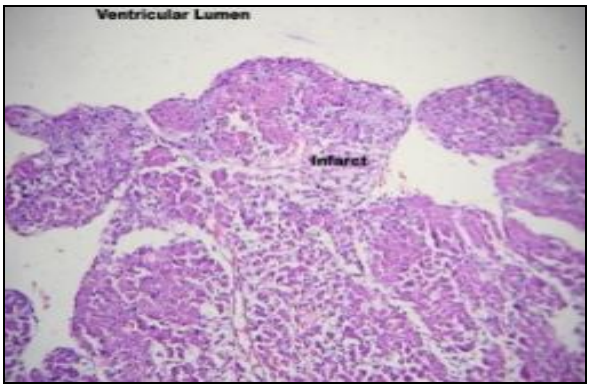

FIGURE 8: GROUP 4 BTE DOSE 2

Photomicrograph of BTE dose 2 (i.e. group IV) showed large areas of infarcted cardiac muscle tissue with extensive loss of muscle fibers, scattered inflammatory cells and isolated residual muscle fibers, thus offering no protection as compared to the Toxic control group (i.e. group II).

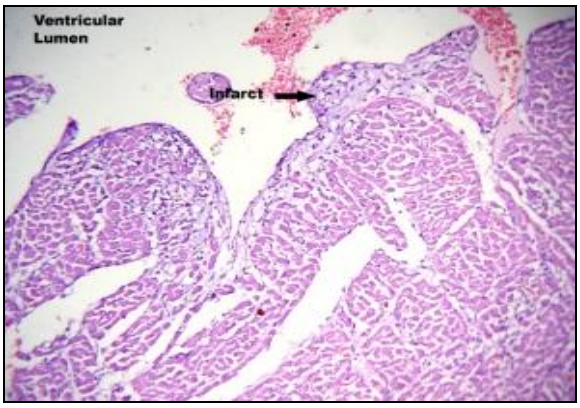

FIGURE 9: GROUP 5 BTE DOSE 3

Photomicrograph of BTE dose 3 (i.e. group V) showed large areas of infarcted cardiac muscle tissue with loss of muscle fibers, scattered inflammatory cells and isolated residual muscle fibers, thus offering no protection as compared to the Toxic control group (i.e. group II).

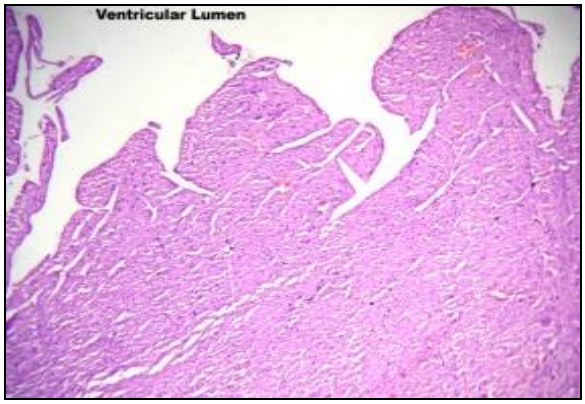

FIGURE 10: GROUP 6 BTE DOSE 3 PER SE

Photomicrograph of BTE dose 3 *per se* group (i.e. group VI) revealed normal cardiac fibers and regular morphology and showed muscle fibers with striations in cytoplasm and elongate nuclei.

FIGURE 11: GROUP 7 STANDARD

Photomicrograph of Standard group (i.e. group VII) revealed very small infarcted cardiac muscle tissue, although it showed loss of muscle fibers, scattered inflammatory cells in the infracted area.

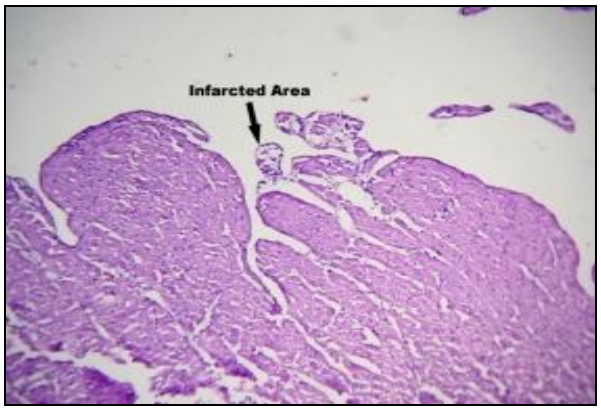

FIGURE 12: GROUP 8 STANDARD + BTE DOSE 3

Photomicrograph of standard + BTE dose 3 group (i.e. group VIII) showed very small infracted cardiac muscle tissue, although it showed loss of muscle fibers, occasional inflammatory cells and isolated residual muscle fibers.

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