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Research Article

Cardioprotective Activity of *Hybanthus Enneaspermus (Linn.)* On Isoproterenol Induced Rats

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ABSTRACT
Myocardial infarction (MI) is the interruption of blood supply to part of the heart, causing heart cells to die, commonly due to occlusion (blockage) of a coronary artery. Herbal drugs are known to exhibit creditable medicinal properties for the treatment of heart ailments and need to be explored to identify their potential application in prevention and therapy of human ailments. Considering this aspect, the study aimed to elucidate the cardio protective activity of *Hybanthus enneaspermus*. Myocardial infarction was induced by a subcutaneous administration of isoproterenol. The positive inotropic and chronotropic response of isoproterenol caused a severe oxidative stress in the myocardium through increased lipid per oxidation. *Hybanthus enneaspermus* was administered at a dose of 500 mg/kg, orally for 4 weeks. On days 29 and 30, the rats in the isoproterenol control and *Hybanthus enneaspermus* treatment groups were given isoproterenol (20mg/100g), subcutaneously at an interval of 24 hr. On day 31, haemodynamic parameters were recorded and the hearts were subsequently removed and processed for histopathological and biochemical studies. Histological examination of rat's heart section confirmed myocardial injury with isoproterenol. Administration of plant extract of *Hybanthus enneaspermus* reduced the oxidative stress by decreased lipid per oxidation and reduced glutathione (GSH) and also normalized the levels of cardiac marker enzymes such as CK, LDH, SGOT, SGPT and cardiac specify protein Troponin I in the blood of both group-I normal and group III isoproterenol myocardial infarcted rats treated. *Hybanthus enneaspermus* -treated animals showed a lesser degree of cellular infiltration in histopathological studies.

INTRODUCTION
Myocardial infarction (MI) means that part of the heart muscle suddenly loses its blood supply. Without prompt treatment, this can lead to damage to the affected part of the heart. An MI is called a heart attack or a coronary thrombosis (Thygesen *et al*., 2007) There are different types of MI. The two main types are called ST elevation MI (STEMI) and non-ST elevation MI (NSTEMI) (Montalescot *et al*., 2007). In a STEMI, the artery supplying an area of the heart muscle is completely blocked. In a NSTEMI, the artery is only partly blocked (Lamas *et al*., 2010). The common cause of an MI is a blood clot (thrombosis) that forms inside a coronary artery, or one of its branches. This blocks the blood flow to a part of the heart (Thygesen *et al*., 2007). The onset of symptoms in myocardial infarction (MI) is usually gradual, over several minutes, and rarely instantaneous. Classical symptoms of myocardial infarction include acute coronary syndrome, chest pain, shortness of breath, nausea, vomiting, palpitations, sweating, anxiety or a feeling of impending doom (Thygesen *et al*., 2007). Risk factors for myocardial infarction includes smoking, hypercholesterolemia, hyperlipoproteinemia, high low density lipoprotein and low high density lipoprotein, Diabetes, High blood pressure (Khader *et al*., 2003) , Older age, Obesity (Yusuf *et al*., 2005). Complications of myocardial infarction (MI) include Arrhythmias, Congestive Heart Failure, Cardiogenic Shock, Ventricular Aneurysm, Pericarditis, Dressler Syndrome and Pulmonary Embolism (Weir *et al*., 2006).

Oxidative stress is a condition in which oxidant metabolites exert their toxic effect because of an increased production or an altered cellular mechanism protection (Block *et al*., 2002). Increased oxidative stress and the generation of the free oxygen radicals can result in modification of LDL to oxidized LDL that could lead to atherosclerotic lesions. Also, inflammation occupies a very important central position in all phases of atherosclerosis, which is underlying cause of myocardial infarction (Libby, 2003). As oxidative stress appears to be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied. Antioxidants have gained popularity recently for their many health benefits. They have been shown to lower the risk of heart disease (Devasagayam *et al*., 2006). The commonly prescribed drugs to prevent a further MI are, aspirin to reduce the 'stickness' of platelets in the blood, clopidogrel, a beta-blocker drug to reduce the developing of abnormal heart rhythms, an

ACE inhibitor drug having a protective effect on the heart, a statin drug to lower the cholesterol level in the blood. This also helps to prevent the build-up of atheroma (Thygesen *et al*., 2007). Apart from these drugs, a large number of epidemiological studies show that diets rich in fruits and vegetables which are rich in antioxidants are associated with lower incidence of Cardio Vascular Diseases (CVDs) (Scartezzini and Speroni , 2000). Similar to these dietary sources, Indian medicinal plants are also known for their cardio protective properties and are rich sources of antioxidants (Jadhav and Bhutani, 2002, Kirtikar and Basu, 1988).

Hybanthus enneaspermus (Violaceae), is a perennial herb commonly named as**"***Orithal thaamarai"* in Tamil and *Rathnapurusha* in Sanskrit. The herb is used as a cardio tonic. Decoction of leaves and tender stalks is demulcent. The fruit is used to treat scorpion sting (Boominathan *et al*., 2003). The plant has been reported to have anti-inflammatory, antitussive, antiplasmodial, anticonvulsant and free radical scavenging activity (Nichans and Samuelson, 1968). The plant is reported to contain aurantiamide acetate, isoarborinol, b-sitosterol and triterpene. Due to the lack of experimental evidence on the biochemical role of Hybanthus enneaspermus on myocardial infarction induced by isoproterenol (ISPH), the study was carried out to elucidate the cardio protective activity of extract of *Hybanthus enneaspermus* plant.

MATERIALS AND METHODS

Male albino Wister rats weighing about 120-180g were used in the study. The animals were housed in polypropylene cages and maintained in controlled temperature with 12hrs period of light and dark and fed with standard rat feed and water. The animals were grouped as group-I (normal) rats, group-II isoproterenol induced myocardial infarcted rats without treatment (Control) and group-III isoproterenol induced myocardial infarcted (treated) with plant extract of *Hybanthus enneaspermus* 500mg/kg body weight per day for 20days.Myocardial infarction was induced by a subcutaneous administration of isoproterenol (20mg/100g) twice at an interval of 24hrs. *Hybanthus enneaspermus* plant was collected, shade dried and soaked with ethanol (70%) for 48hrs and a semisolid extract was used as drug, after complete elimination of ethanol under reduced pressure. *Hybanthus enneaspermus* was administered at a dose of 500 mg/kg, orally for 4 weeks. On days 29 and 30, the rats in the isoproterenol control and *Hybanthus enneaspermus* treatment groups were given isoproterenol (20mg/100g), subcutaneously at an interval of 24 hr. On day 31, haemodynamic parameters were recorded and the hearts were subsequently removed and processed for histopathological and biochemical studies. Histological examination of rat's heart section confirmed myocardial injury with isoproterenol. After the experimental period, the rats were scarified by cervical decapitation. The heart was dissected out, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). Homogenate was centrifuged and supernatant was used for the assay of glutathione and lipid peroxides in serum and heart homogenate. The collected samples (Serum & homogenate) were used for analysis of different biochemical parameters and assay of marker enzymes. Three parameters and four marker enzymes were analyzed and the methods used for analysis were LPO[Malondialdehyde] (Reitmann and Frankel,1957), GSH(Reduced Glutathione), serum Troponin (Varley *et al*., 1984), serum GOT(Glutamate Oxaloacetate transferase), serum GPT(Glutamate Pyruvate transaminase) (King, 1959), and serum CK(Creatine kinase) (Tilak Jain and Devasagayam, 2006), serum LDH(Lactate Dehydrogenase) (Heber, 2001). In order to determine the myocardial necrosis by direct staining the myocardium of rat was frozen immediately after removal. When the tissue was firm, the heart was sliced into 3 - 5 mm thick slice from the apex toward the atrioventricular groove and incubated in 1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) in phosphate buffer saline with pH 7.4 at 37 ^{o} C for 20 min. The sections were examined under light and photographs were taken (Khalil *et al*., 2006).

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. (Zhou *et al*., 2008).The results were presented as mean $\pm$ standard deviation (SD). Student't' was used to analyze statistical significance.

RESULT AND DISCUSSION

Myocardial infarction remains a leading cause of morbidity and mortality worldwide. Prompt treatment of a heart attack is indispensable to prevent permanent damage and to save the life. In the traditional Indian medicinal system, a major role has been played by the herbal plants especially, in the aspect of cardio protection. In this context, there is a need to reveal the cardio protective activity of extract of *Hybanthus enneaspermus* plant. Several herbs and herbal products have been recommended to promote a healthy heart. The prophylactic and therapeutic effects of many plant extracts in reducing cardiovascular diseases (CVDs) have been reviewed. These include *Allium sativum*(garlic), Allium cepa(onion),*Curcuma longa* (turmeric),*Emblica officinalis*(amla), *Momordica cymbalaria* (Athalakkai), *Mangifera indica*(Mango), *Daucus carota*(wild carrot), *Punica granatum*(Pomegranate),*Piper longum*(Long pepper), Ocimum sanctum(tulsi),*Withania somnifera* (ashwagandha), *Zingiber officinalis*(ginger). These plants exhibit potent antioxidant effects, which might be the mechanism behind their beneficial therapeutic properties (Tilak Jain and Devasagayam, 2006). Moreover, guggulipid, tocotriends derived from palm oil, soyaprotein, isofalvones and Chinese red yeast rice have been shown to lower cholesterol levels by different mechanisms. Other antioxidant rich and angiogenic herbs such as green tea, black tea and red wine have the potential to reduce the progression of atherosclerosis (Heber, 2001). Free radicals and reactive oxygen species have been implicated in cardiac diseases and metabolic disorders, which result due to exposure to chemicals and environmental agents.

In our study Isoproterenol (ISPH) is used to induce myocardial damage. Isoproterenol (ISPH), a synthetic catecholamine and beta- adrenergic agonist, has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle and is also well known to generate free radicals and stimulate lipid per oxidation, which may be a causative factor for irreversible damage to the myocardial membrane in experimental myocardial infarction (Senthil Kumar *et al*., 2001). Catecholamines rapidly undergo auto-oxidation and it has been suggested that the oxidative products of catecholamines are responsible for changes in the myocardium (Yates and Dhalla, 1975). High concentrations of catecholamines have been reported to cause necrotic lesions in the heart resulting in myocardial infarction in experimental animals (Knufman *et al*., 1987). *Hybanthus enneaspermus* is said to be one of the effectual herbal plant due to the eminent medicinal aptitude it possess. The herb is used as a diuretic, demulcent and cardio tonic and it is also considered to be extremely beneficial to men and improves sexual potency. In Ayurveda it is known as Sthalakamala. The root is diuretic and is used in urinary affections and bowel complaints of children. In folklore the plant is used in case of pregnant and parturient women, and in case of gonorrhoea and urinary infections. An infusion of the plant extract is given in case of cholera (Kirtikar and Basu, 1975). The observations made on different groups of normal, experimental and treated animals were discussed. 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). In our study, we have noted reduced levels of serum CK (Fig. 1), LDH (Fig. 2), SGOT (Fig. 3) and SGPT (Fig. 4) of isoproterenol myocardial infarcted rats where as in homogenate they were increased. Pretreatment with mangiferin, (from the leaves of *Mangifera indica*) (5, 10 and 20mg/100 g body weight, daily) (Group 4) retained the activities of these enzymes to near normal levels (P<0.001) in heart tissue as compared to (Group 2) ISPH myocardial infracted rats (Suchalatha and Shyamala Devi, 2004). Mangiferin, a principal phenolic compound also has potent free radical scavenging activity and protective effect against altered changes in AST and ALT activities caused by toxicant (Yoshikawa *et al*., 2002). Pretreatment with ethanolic extract of *M. cymbalaria* at 250 and 500 mg/kg prevented the elevation of serum marker enzymes, lactate dehydrogenase (LDH), transaminases (SGOT,SGPT), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) caused by isoproterenol (ISPH)(60 mg/kg s.c, 2days)- induced myocardial infarction in rats (Waring *et al*., 2000).Pretreatment with butanolic fraction of *Punica granatum* seed juice extract (100 mg/kg, p.o. and 300 mg/kg, p.o.) significantly reduced (P < 0.05) the activities of CK,LDH and transaminases (SGOT,SGPT) as compared to Isoproterenol (85 mg/kg) treated rats (Basu and Penugonda, 2009). Muruganandan *et al*., 2002 have reported that IP administration of mangiferin (*Mangifera indica*) significantly reduce the activity of CK and LDH in heart as well as ameliorates the oxidative stress. Treatment with *Daucus carota* (250 mg/kg and 500 mg/kg dose) extract showed significant increase (p<0.001) in lactate dehydrogenase level, when compared to isoproterenol treated groups (Prabhu *et al*., 2006). Pretreatment with ethanolic extract of *M. cymbalaria* at 250 and 500 mg/kg prevented the

elevation of serum marker enzyme, creatinine kinase-MB Fraction (CK-MB) caused by isoproterenol (ISPH) (60 mg/kg s.c, 2days) induced myocardial infarction in rats (Waring *et al*., 2000).

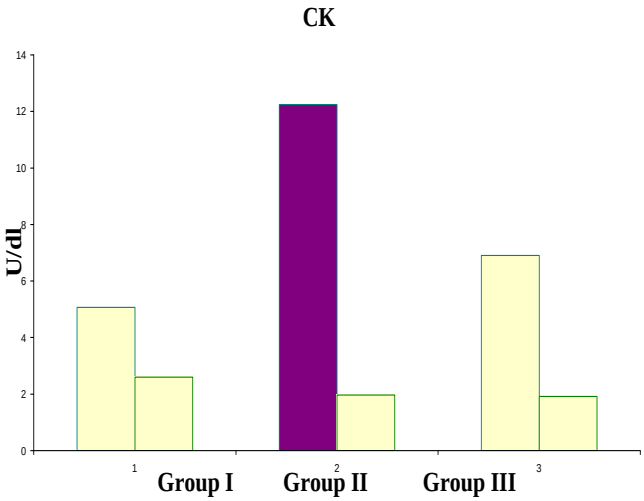

Figure 1: Effect of Plant Extract of *Hybanthus Enneaspermus* on Serum & Homogenate Level of CK in Normal and Experimental Rats

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Pretreatment of *Withania somnifera* at 50 mg/kg dose on isoproterenol (85 mg/kg) induced myocardial rats showed a significant decrease in creatinine kinase and lactate dehydrogenase (P<0.01) levels (Jennings *et al*., 1990). Pretreatment with ethanolic *zingiber officinale* extract (200 mg/kg) in isoproterenol (ISPH)-treated rats showed a near normal (*P*<0.01) activity of the diagnostic marker enzymes LDH and CK, in the serum. (Sheela and Shyamala Devi, 2000).

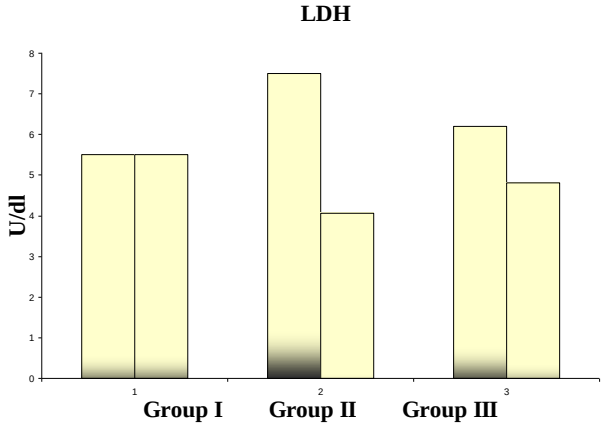

Figure 2: Effect of Plant Extract of *Hybanthus Enneaspermus* on Serum & Homogenate Level of LDH in Normal and Experimental Rats

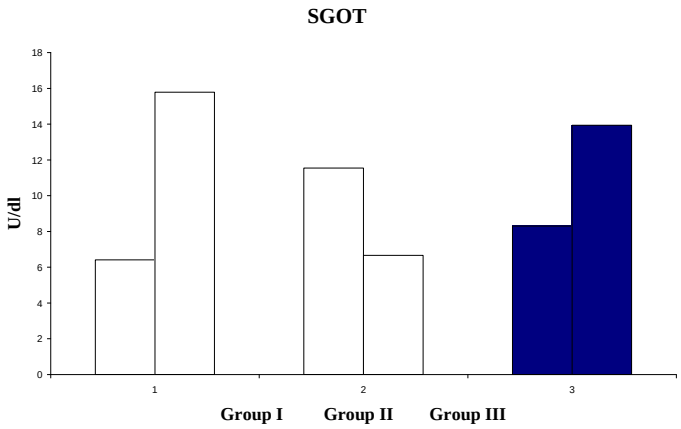

Figure 3: Effect of Plant Extract of *Hybanthus Enneaspermus* on Serum & Homogenate Level of SGOT in Normal and Experimental Rats

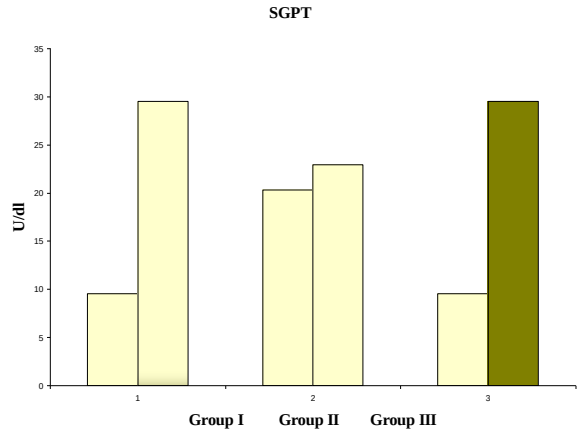

Figure 4: Effect of Plant Extract of *Hybanthus Enneaspermus* on Serum & Homogenate Level of SGPT in Normal and Experimental Rats

We have noted a significant decrease in LPO, GSH, and TROPONIN level (Table 1) where as in homogenate the levels were vice-versa in both normal and isoproterenol myocardial infarcted rats treated with plant extract of *Hybanthus enneaspermus*. Lipidperoxide is an important pathogenic event in myocardial infarction and the accumulated lipid peroxides reflects the various stages of the disease and its complications (Grylewski, 1980).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 (Jayalakshmi and Niranjali Devaraj, 2004). In our study

the reduction noticed in the level of GSH and LPO in serum and heart of ISPH induced myocardial infarction was either due to increased degradation or decreased synthesis of glutathione. Garlic oil (75 mg / k.g b.w) for 60 days pretreated isoproterenol (20 mg/kg b.w) administered rats maintained the level of lipid peroxides to near normal ($p < 0.05$)when compared to control (Saravanan and Prakash, 2004)..Treatment with *Daucus carota* extract at 250 mg/kg and 500 mg/kg doses showed significant decrease ($p<0.01$) in lipidperoxidation level, when compared to isoproterenol (5.25 mg/kg and 8.5 mg/kg) treated group (Allard *et al*., 1994). Isoproterenol-intoxicated rats showed a significant decrease in glutathione levels in heart and serum. Oral administration of *Allium sativum* (garlic oil) (75 mg / k.g b.w) for 60 days along with isoproterenol (20 mg/kg b.w) intoxicated rats maintained the concentration of GSH at near normal ($p < 0.05$) levels (Jayalakshmi and Niranjali Devaraj, 2004). Administration of petroleum ether extract of roots of *Piper longum* (100 mg kg^{-1})helps to minimize the lipid peroxide values in rats treated with isoproterenol (20 mg/100 g) and maintains glutathione levels to near normal ($p < 0.05$) levels (Sasikumar and Devi, 2000). Pre-treatment of hydroalcoholic extract of *Ocimum sanctum* (Os) at different doses (25, 50, 75, 100, 200 and 400 mg/kg) against isoproterenol (200 mg/kg) induced showed a significantly reduced serum glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) (Sharma *et al*., 2001). *Ocimum sanctum* (Os) at the dose of 25, 50, 75 and 100 mg/kg reduced significantly glutathione (GSH), It also inhibited the lipid peroxidation as observed by the reduced thiobarbituric acid reactive substances (TBARS) and cardiac troponin levels against isoproterenol (200 mg/kg) induced myocardial infarction in rats (Priscilla and Prince, 2009). degree of unstained region compared to ISPH -treated group (Figure 5). In histopathological examination, normal architecture was observed in control animals whereas animals treated with ISPH showed thrombus formation, contraction band necrosis and inflammation. Animals pretreated with plant extract (500 mg/kg) revealed much less intensity of the above changes (Figure 6). It has been reported that, TTC forms a red formazan precipitate with LDH of the viable myocardial tissue in the presence of mitochondrial dehydrogenase enzyme system, whereas areas of necrosis lack mitochondrial dehydrogenase activity and do not stain. Consequently, areas not stained with TTC correspond to areas of total necrosis. (Lie *et al*., 1975).

On histopathological examination, ISPH -treated group, demonstrated thrombus formation, contraction band necrosis and inflammation. Pretreatment with plant extract of *Hybanthus enneaspermus* reversed these changes. It is concluded that plant extract of *Hybanthus enneaspermus* has a potential to inhibit the cardio toxic effects induced by ISPH and possesses a significant medicinal value in the prophylactic treatment of MI.

Table 1: The results for LPO, GSH, and TROPONIN were summarized in a table

Parameters (mg/dl)	Group-I	Group-II	Group-III
Serum LPO	22.738 ± 3.160	38.06 ± 19.303**	21.906 ± 3.021*
Homogenate LPO	10.476 ± 5.661	23.214 ±20.009**	10.593 ± 3.284*
Serum GSH	5.59 ± 0.050	3.845 ± 0.069**	5.586 ± 0.050*
Homogenate GSH	53.200 ± 0.19	30.166 ± 0.55**	41.400 ± 0.19*
Serum TROPONIN	3.7 ± 0.7	5.6 ± 0.99**	3.9 ± 0.5*

** *P < 0.001 significantly different from group – I rats.*
* *P < 0.001 significantly different from group – II rats.*

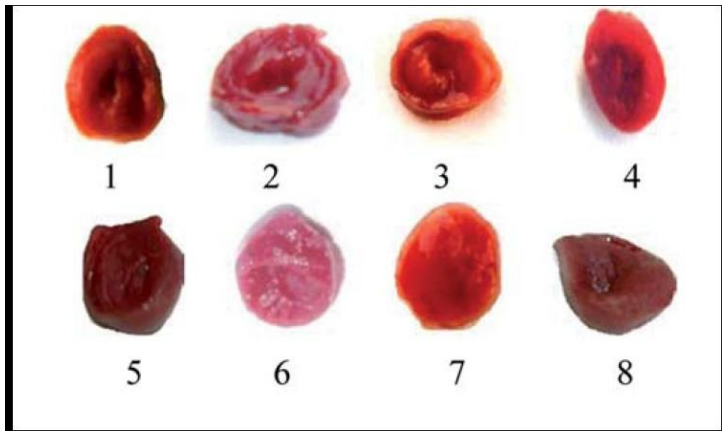

Figure 5: Photographs of transverse sections of heart from control and experimental groups stained by triphenyltetrazolium chloride 1: group1; 2: group 2; 3: group 3; 4: group 4; 5: group 5; 6: group 6; 7: group 7; 8: group 8.

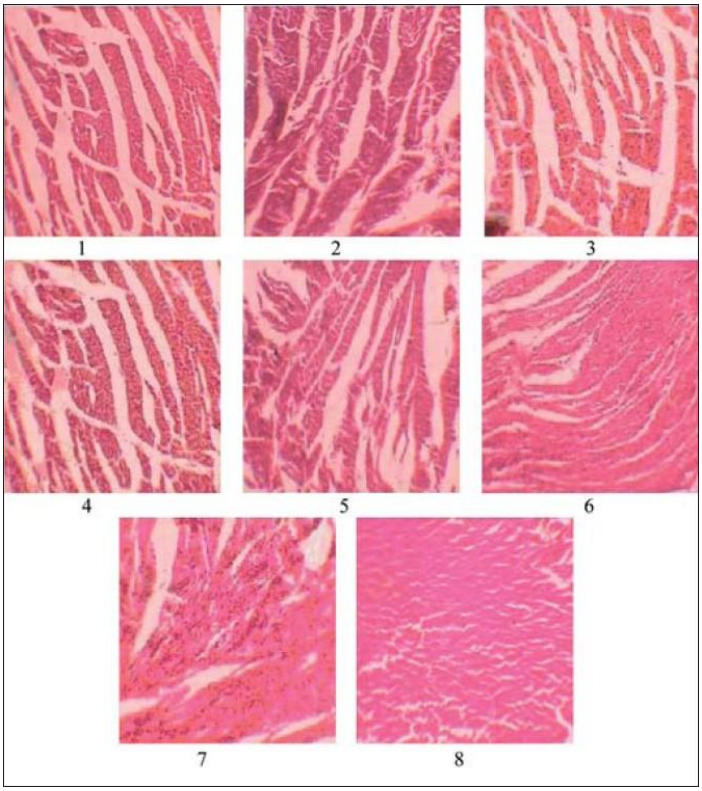

Figure: 6 Photomicrographs of histopathological examination (10×) of the heart from control and experimental groups Section of the heart from 1 (group-1) shows normal architecture. Section of the heart from 2 (group 2) reveals thrombus formation, contraction band necrosis and inflammation. Sections of heart from 3 (group 3); 4 (group 4) and 5 (group 5) shows normal architecture, whereas. Sections of heart from 6 (group 6); 7 (group 7) and 8 (group 8) shows less intensity of congestion, rombus formation and necrosis.

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