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CARDIOPROTECTIVE EFFECT OF AESCIN ON ANTIOXIDANT AND LIPID PROFILE IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN RATS

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Keywords:

Aescin, Antioxidants, Isoproterenol, Lipids, Myocardial damage

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ABSTRACT: The efficacy of aescin on the liver and cardiac markers, lipid profile, and antioxidant status in rats with myocardial infarction (MI) induced by isoproterenol (ISO) was investigated in this study. Three doses of aescin (5, 10, and 20 mg/kg of b.w) were administered to rats for the first 21 days. After the treatment period, ISO (60 mg/kg of b.w) was given subcutaneously to the rats on the 22nd and 23rd day. Cardiovascular and hepatic markers (CK, ALT, CK-MB, AST, cTnI, and cTnT) has been analyzed to investigate cardiac and liver damage. The activities of antioxidant enzymes (CAT, GST, SOD, and GPx) were decreased in both cardiac tissue and erythrocytes of ISO rats. The levels of phospholipids (PLs), total cholesterol (TC), free fatty acids (FFA), and triglycerides (TG) were increased significantly in the serum of the rats administrated with ISO. The results of the present study implies that aescin pretreatment reduces oxidative stress and exhibits cardioprotective action by scavenging the free radicals and maintaining the levels of circulatory and cardiac lipids. Hematoxylin and eosin staining method was used to examine the cardiac histological changes in the experimental rats. The results showed that ISO-administered rats pretreated with aescin reduced cardiac tissue damage compared with ISO-alone injected rats.

INTRODUCTION: Myocardial infarction (MI) is a severe health concern in developed and developing countries, affecting a large proportion of the global population and significantly impacting mortality numbers [1]. It is a clinical disorder caused by a sustained restriction of cardiac blood flow, and this mechanism can occur even in minor blockages when cholesterol plaque is disrupted [2]. The cardiac markers detection in serum is essential in the early diagnosis of MI.

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Troponins (cTns), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and creatine kinase MB (CK-MB) are used as the cardiac marker for the diagnosis of myocardial damage. The excellent specificity of cTns among cardiac markers makes them the ideal standard for MI diagnosis. The level of cTns in the serum is proportional to the size of the infarct area [3].

Reactive oxygen species (ROS) is a byproduct of aerobic metabolism. Still, either too much ROS or less antioxidants can cause oxidative stress, damaging the DNA, lipids and proteins in the cell [4]. The antioxidant defense system is a primary defense mechanism against the damage caused by oxidation [5]. Antioxidants in food can stop the harmful effects of ROS by preventing the

imbalance between the removal and production of ROS *via* scavenging the excess ROS. Natural products are in high demand worldwide because of their better and safer effects against various diseases, including cardiovascular diseases (CVDs) caused by oxidative stress [6]. A high level of lipids in the blood is a substantial risk factor for MI. Lipid levels in the bloodstream should be reduced while lipoprotein levels should be maintained. A large concentration of free radicals promotes the deposition of cardiac lipids, which promotes lipid peroxidation and causes irreparable damage to the myocardial membrane [7].

ISO-induced MI is a conventional model for studying cardiac functions and the therapeutic effects of various medications. Experiments on animals after the administration of ISO have shown similar alterations in the heart's metabolism, pathophysiology, and morphology to those seen in patients with MI. Therefore, in this study we have used ISO to cause MI in rats.

ISO is a synthetic catecholamine that can induce MI in rats by producing infarct-like necrosis in the cardiac muscle [8]. The production of cytotoxic free radicals during the autoxidation of ISO is one of the most vital causes of ISO-induced MI. The harmful effects of ISO are due to the synthesis of adrenochrome and the conversion of quinones following the oxidation of hydroxyl groups in ISO [9]. Oxidative metabolites of ISO and adrenochrome induce contractile failure and cellular necrosis in the rat myocardium. During the oxidation of ISO, highly hazardous oxygenated free radicals are produced, which are harmful to the proteins and intracellular enzymes. In addition, free radicals may induce membrane peroxidation, resulting in structural and functional heart muscle damage [10].

ISO elevates cardiac cAMP levels by activating adenylate cyclase through a G protein-coupled receptor, enhancing the heart's intracellular calcium concentration and altering the signalling cascade [11]. Myocardial Ca^{2+} content increases due to the activation of cAMP-dependent protein kinase (PKA), which phosphorylates the Ca2+channel proteins. This process increases contractile force and myofibrillar overstimulation, which activates Ca^{2+} dependent phospholipases and protease enzymes and causes increased oxygen requirement and excessive ATP depletion, all of which contribute to cardiac muscle cell injury [12].

The natural products from the plants, such as their bark, fruits, stems, leaves, dietary supplements, food, timber, and herbs, are beneficial to increase the antioxidant level, thereby preventing various diseases [13]. The horse chestnut (*Aesculus hippocastanum* L.) is a fast-growing ornamental tree. It is extremely suited to grow in a polluted environment and is used to prevent numerous ailments [17]. In traditional medicine, *A. hippocastanum* has been used as a remedy for bronchitis, diarrhea, venous issues, and hemorrhoids [14]. Aescin is the main bioactive ingredient of *Hippocastanum* L, chemically related to triterpene glycoside [15]. **Fig. 1** depicts the chemical structure of the aescin found in horse chestnuts. Aescin has several pharmacological properties, including anti-edematous, anti-inflammatory, anti-cancer, and antioxidant properties [16, 17]. A thorough review of the literature revealed no studies on the impact of aescin on ISO-induced MI in rats. Therefore, in this study, we planned to explore the cardioprotective effect of aescin on ISO-induced changes in body weight, antioxidants, cardiac markers, and lipids and histopathological changes in albino Wistar rats.

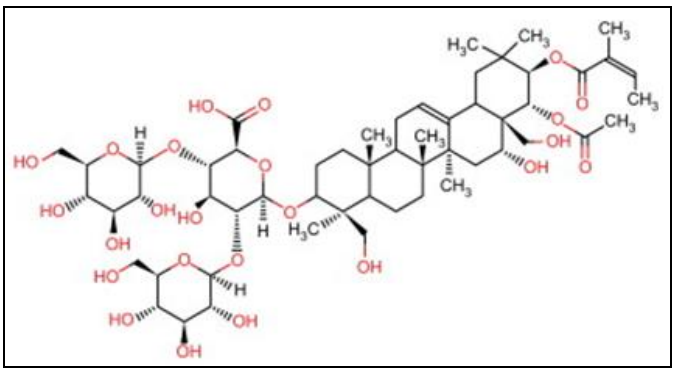

FIG. 1: STRUCTURE OF AESCIN

MATERIALS AND METHODS:

Chemicals and Drugs: ISO and aescin were purchased from Sigma-Aldrich (St. Louis, MO). CK,ALT, LDH, CK-MB, and AST were assayed using kits acquired from Agape diagnostics (Kerala, India) and Qualigens Diagnostics (Mumbai, India). cTnI and cTnT were purchased from Reckon Diagnostics Pvt. Ltd Gujrat, India. All other chemicals used were of analytical grade obtained from E. Merck and Himedia, India.

Animals: Male albino Wistar rats (body weight between 160 and 180 g) were used in this investigation. They were purchased from Biogen, Bangalore. Rats were maintained as per the principles and guidelines of the ethical committee for animal care, Annamalai university, in accordance with the Indian National Law on Animal Care (160/PO/ReBi/S/1999/CPCSEA, Pro. No. AU- IAEC-1224 /1/ 19/PO). The animals were housed in plastic cages with paddy husk for bedding at a temperature of 27 ± 2ºC with 12-hour light: dark cycles. The experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Rats."

Formulation and Administration of AESCIN: After dissolving aescin in distilled water, it was administered post-orally (p.o.) by intubation once a day for 21 days [18].

Induction of MI: Experimental rats were given a subcutaneous injection of 60 mg/kg body weight of ISO after being dissolved in saline for two continuous days to induce MI [19].

Experimental Protocol: The animals were randomized to 6 groups of 8 rats each and treated as follows:

48 animals / 6 group (n=8)

Group I	:	Normal
Group II	:	Aescin (20 mg/kg b.w, p.o for first 21 days)
Group III	:	Isoproterenol (60 mg /kg b.w, s.c. on 22nd and 23rdday)
Group IV	:	Aescin (5 mg /kg b.w, p.o first 21 days) + ISO (60 mg /kg b.w, s.c. on 22nd and 23rdday)
Group V	:	Aescin (10 mg /kg b.w, p.o for first 21 days) + ISO (60mg/kg b.w, s.c. on 22nd and 23rdday)
Group VI	:	Aescin (20 +mg /kg b.w, p.o for first 21 days) + ISO (60 mg /kg b.w, s.c. on 22nd and 23rdday)

The body weight of the rats was only recorded once during the experimental period. Ketamine hydrochloride (60mg/kg b.w.) was utilized (intramuscular injection) as an anesthetic agent prior to the animal sacrifice. Following the second dose of ISO injection, the rats were sacrificed by cervical dislocation. Following the animal sacrifice, blood was drawn from a jugular vein, and the serum was separated by centrifugation. The thoracic cavity was opened immediately after the collection of blood samples, and the heart was removed, normal saline was used to wash and filter paper was used to blot dry, the organ was weighed. The tissues were stored in liquid nitrogen (-80 °C) to analyze different biochemical markers.

Biochemical Estimations:

Processing of Cardiac Tissue: The heart tissue samples were subsequently homogenized using RQ 127A, REMI Motors (Mumbai, India) in 25 mM of phosphate buffer(pH 7.4) to obtain a homogenate roughly 10% weight by volume. After 10 minutes of centrifugation of the homogenate at 2000 rpm, the supernatant was collected for the biochemical assay. The cardiac tissues were also preserved in buffered formalin for histological examinations.

Assessment of Cardiac Marker Enzymes: Activities of LDH, CK-MB, CK, ALT and AST in serum were determined using Agape diagnostics and Qualigens Diagnostics kits as per the manufacturer's procedure. All of these marker enzyme activities were reported in IU/L. The amounts of cTnI and cTnT in serum were quantified using standard kits (Roche diagnostics).

Assessment of Enzymatic Antioxidants: The Kakkar *et al.* [20] method was used to measure the SOD activity in the erythrocyte and heart. Formazon blue is formed when superoxide radicals combine with nitroblue tetrazolium in the presence of decreased nicotinamide adenine dinucleotide (NAD). SOD prevents the formation of formazon blue by removing superoxide radicals. The color

intensity was read at 560 nm, it is inversely related to the activity of the enzyme. Using Sinha *et al.* [21] approaches, the catalase activity in the myocardium was measured. In this process, heated dichromate present in acetic acid transforms into perchromic acid and then chromic acetate when it react with hydrogen peroxide. The formed chromic acetate was measured at 620 nm. The Rotruck *et al.* [22] approaches were used to measure GPx activity. GSH and hydrogen peroxide were allowed to react with a defined quantity of enzyme preparation for a predetermined time. Ellman's reaction was used to calculate the GSH content that remained after the reaction. Using the Habig *et al.* [23] techniques, the activity of GST was measured in the heart tissue, 1-chloro-2,4-dinitrobenzene was used as a substrate, and intensity was measured at 340 nm.

Assessment of Lipids: Folch *et al.* 24 method separated the lipids from the tissue samples. 10 mL of a chloroform-methanol (2:1 v/v) mixture with a known sample volume was incubated for 30 minutes and filtered through Whatman filter paper (No. 42, pore size: 8 µm) into a separating funnel. The filtrate was dissolved in 0.2 mL of physiological saline, left in place overnight, and the lower phase lipids were drained. The lipid extracts were re-dissolved in 3.0 mL of a chloroform-methanol (2:1) mixture for lipid analysis (only the aliquots). Zlatkis *et al.* [25] and Fossati and Prencipe [26] techniques were used to determine TG and TC levels in plasma. PLs and FFA were calculated using the methods developed by Hron and Menahan [27] and Zilversmit and Davis [28].

Histological Examination of Heart Tissues: The heart was removed immediately after the sacrifice of the animal and rinsed in saline before being preserved in a 10% neutral buffered formalin solution. The paraffin-embedded fixed tissues were sliced into serial sections. Hematoxylin and eosin were used to stain the tissue for the histopathological analysis, and photomicrographs were taken under a light microscope (100x).

Statistical Analysis: One-way analysis of variance (ANOVA) was used for the statistical analysis, and groups were compared using Duncan's multiple range test (SPSS/17.0). All quantitative measures were expressed as means ± SD for the experimental rats. The results were considered statistically significant if the *P* value was less than 0.05.

RESULT:

Heart and Body Weight Changes in Different Groups of Rats: The heart weight, body weight, and heart weight/body weight ratio were observed in all the experimental and normal rats depicted in **Table 1.** There were no significant variations in the body weight across the groups at the end of this study. In contrast, a substantial increase in heart weight were seen in ISO-injected rats ($P < 0.05$) compared to normal rats. The weight of the heart of ISO rats pretreated with 10 mg/kg b.w of aescin was significantly ($P < 0.05$) reduced than other doses, indicating the cardioprotective action of aescin. No differences were observed in the heart weight of the normal rats and aescin alone treated rats.

TABLE 1: EFFECT OF AESCIN ON BODY WEIGHT, HEART WEIGHT AND HEART WEIGHT/BODY WEIGHT RATIO IN NORMAL AND ISO-INDUCED RATS

Groups/Treatments	Bodyweight (g)	Heart weight (g)	Heart weight/ Bodyweight ratio (%)
Normal	175.66 ± 12.38	0.534 ± 0.03^{a}	0.304 ± 0.01^{a}
Aescin (20 mg/kg b.w)	172.74 ± 12.22	0.518 ± 0.03^{a}	0.300 ± 0.01^{b}
ISO (60 mg/kg b.w)	164.45 ± 11.59	0.887 ± 0.06^{b}	0.539 ± 0.01^{c}
Aescin + ISO (5 mg/kg b.w)	168.94 ± 11.98	0.788 ± 0.05^{c}	0.466 ± 0.01^{d}
Aescin + ISO (10 mg/kg b.w)	174.91 ± 12.33	0.559 ± 0.03^{a}	0.319 ± 0.01^{e}
Aescin + ISO (20 mg/kg b.w)	180.81 ± 12.74	0.607 ± 0.04^{d}	0.335 ± 0.01^{f}

Values are means ± SEM for eight rats. Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT)

Effect of Aescin on Cardiac Markers in the Serum of ISO-induced MI Rats: The effects of aescin on serum LDH, CK, CK-MB, AST and ALT activities and cTnT and cTnI levels in normal and experimental rats were shown in **Fig. 2, 3** and **4**. The activity of cardiac marker enzymes and the levels of cTnI and cTnT were significantly higher($P < 0.05$) in ISO-administered rats than in normal rats. Pretreatment with aescin (5, 10 and 20 mg/kg b.w) on ISO rats prevented these alterations in MI rats. In this study, we observed that the 10mg/kg b.w of aescin was more effective than the other two doses.

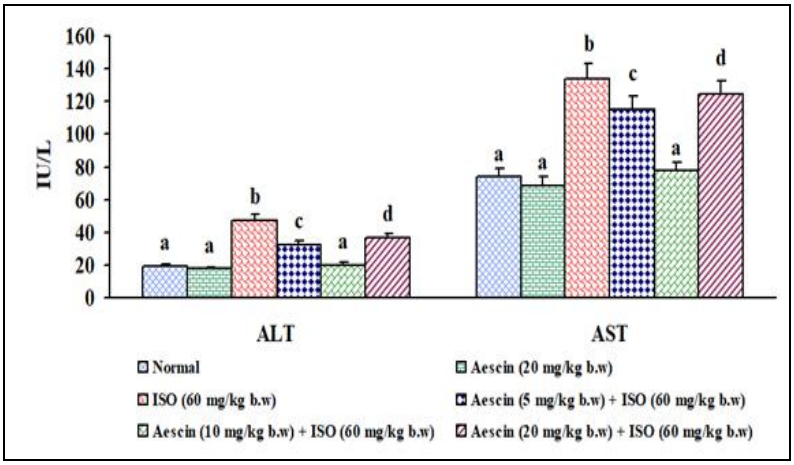

FIG. 2: EFFECT OF AESCIN ON SERUM HEPATIC MARKER ENZYMES ALT AND AST IN ISO-TREATED RATS

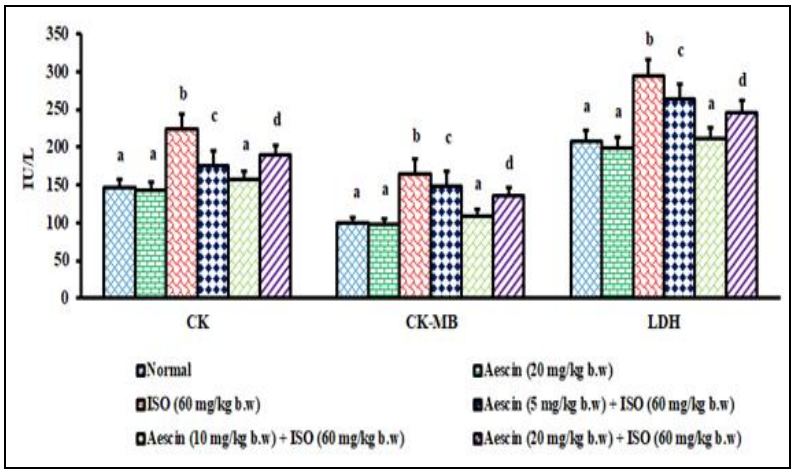

FIG. 3: EFFECT OF AESCIN ON SERUM CARDIAC MARKER ENZYMES CK, CK-MB AND LDH IN ISO-TREATED RATS

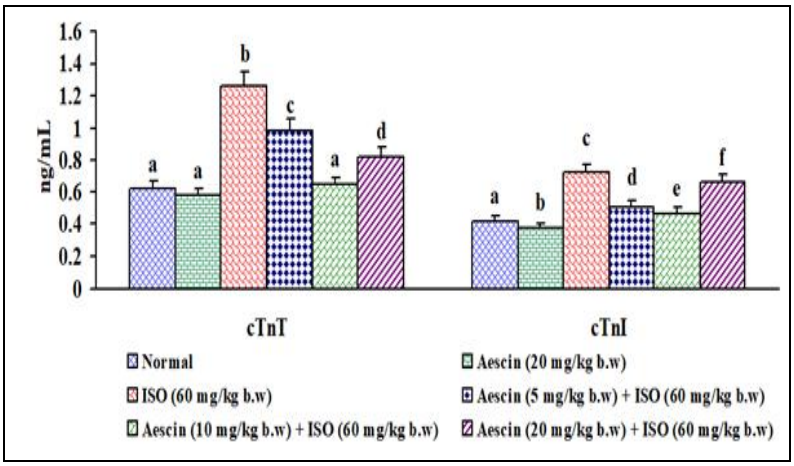

FIG. 4 EFFECT OF AESCIN ON SERUM CARDIAC MARKER cTnT AND I IN ISO-TREATED RATS

Effect of Aescin on Antioxidants in the Erythrocytes and Cardiac Tissue of ISO-Induced MI Rats: The effect of aescin on enzymatic antioxidants such as SOD, GPx, GST and CAT in normal and MI rats is depicted in **Table 2**. When compared to normal rats, ISO significantly ($P < 0.05$) reduced the activities of enzymatic antioxidants in cardiac tissues and erythrocytes of rats. Pre-treated with aescin (5, 10, and 20 mg/kg b.w) before ISO injection significantly ($P < 0.05$) prevented the alterations in the enzymatic antioxidant activities caused by ISO.

TABLE 2: EFFECTS OF AESCIN ON THE ACTIVITIES OF SOD, CAT, GPX, GST IN THE ERYTHROCYTES AND HEART OF NORMAL AND ISO-INDUCED RATS

Groups/Treatments	Normal	Aescin (20 mg/kg b.w)	ISO (60 mg/kg b.w)	Aescin + ISO (5 mg/kg b.w)	Aescin + ISO (10 mg/kg b.w)	Aescin + ISO (20 mg/kg b.w)
SOD Erythrocytes (U*/mg Hb)	9.09 ± 0.63^{a}	8.06 ± 0.57^{b}	5.35 ± 0.37^{c}	7.20 ± 0.50^{d}	9.05 ± 0.63^{a}	6.20 ± 0.43^{e}
Heart (U*/mg Protein)	8.38 ± 0.59^{a}	7.63 ± 0.54^{b}	3.72 ± 0.26^{c}	4.27 ± 0.30^{d}	8.16 ± 0.70^{a}	6.51 ± 0.45^{e}
CAT Erythrocytes (U**/mg Hb)	188.39 ± 13.27^{a}	181.60 ± 12.88^{a}	131.56 ±9.27^{b}	145.87 ± 10.34^{c}	179.49 ± 12.64^{a}	162.90 ± 11.48^{d}
Heart (U**/mg protein)	56.03 ± 3.95^{a}	51.68 ± 3.66^{b}	30.56 ± 2.15^{c}	39.12 ± 2.77^{d}	53.25 ± 3.75^{a}	47.74 ± 3.36^{e}
GPx Erythrocytes (U@ /mg Hb)	14.19 ± 1.00^{a}	13.22 ± 0.93^{b}	8.98 ± 0.63^{c}	10.32 ± 0.73^{d}	13.61 ± 0.95^{a}	12.10 ± 0.85^{e}
Heart (U@/mg protein)	7.20 ± 0.50^{a}	6.37 ± 0.45^{b}	3.85 ± 0.27^{c}	4.32 ± 0.30^{d}	6.82 ± 0.48^{a}	5.19 ± 0.36^{e}
GST Erythrocytes (U$/mg Hb)	7.87 ± 0.55^{a}	7.30 ± 0.51^{b}	5.05 ± 0.35^{c}	5.57 ± 0.39^{d}	6.76 ± 0.47^{e}	6.18 ± 0.43^{f}
Heart (U$/mg protein)	5.97 ± 0.41^{a}	5.69 ± 0.40^{a}	3.50 ± 0.24^{b}	4.13 ± 0.29^{c}	5.29 ± 0.37^{d}	4.71 ± 0.33^{e}

Values are means ± S.D for eight rats. Values not sharing a common superscript differ significantly at $p \leq 0.05$ (DMRT). U* = Enzyme concentration required for 50% inhibition of NBT reduction/min. U** = mmole of hydrogen peroxide consumed/min. U@ = mmole of GSH utilized/min. U$ = mg of CDNB conjugate formed/min

Effect of Aescin on Plasma Lipids in ISO-Induced MI Rats: ISO-induced rats show an increased concentration of TGs, FFA, TC and PLs in the plasma are shown in **Fig. 5** and **6**. Animals that received different doses of aescin (5, 10 and 20 mg/kg b.w) significantly ($P < 0.05$) reduced the concentrations of TGs, TC, PLs, and FFA in the plasma of ISO-administrated rats.

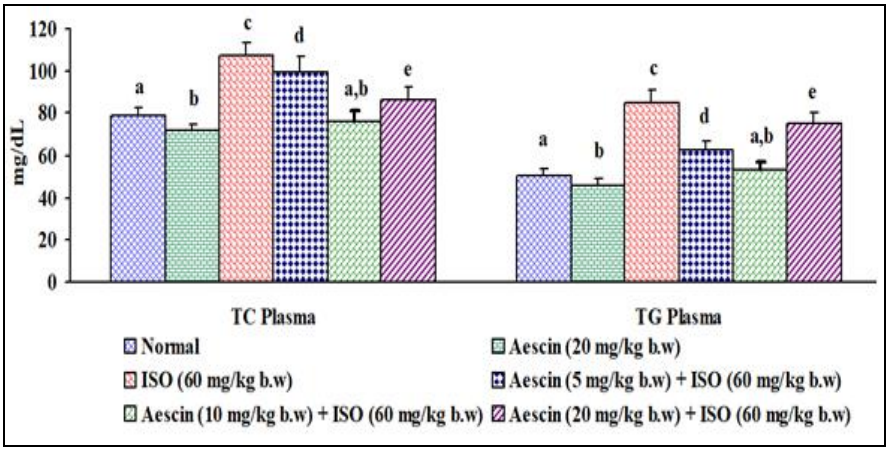

FIG. 5: EFFECT OF AESCIN ON THE CONCENTRATION OF TC AND TGs IN PLASMA OF NORMAL AND ISOINDUCED MI RATS

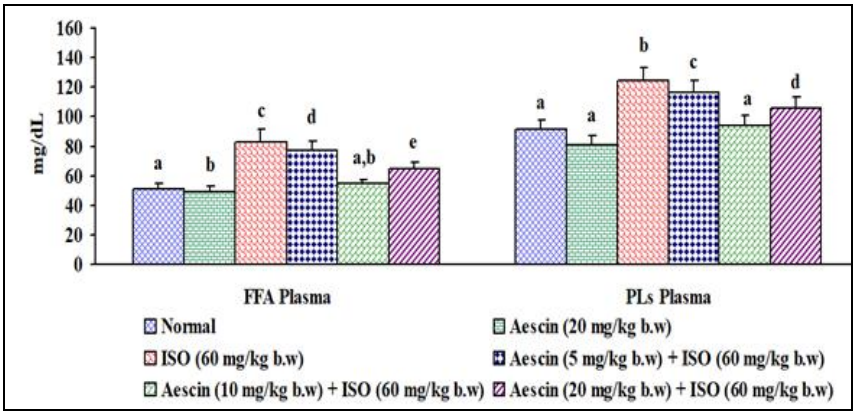

FIG. 6: EFFECT OF AESCIN ON THE CONCENTRATION OF FFA AND PLs IN PLASMA OF NORMAL AND ISO-INDUCED MI RATS

Histopathological Changes in the Heart Tissue of Experimental Rats (Hematoxylin and Eosin 100x): Fig. 7 shows the results of the histopathological investigation of the cardiac tissues of experimental rats. The cardiac tissues of the animals treated with aescin alone and the control animals exhibited normal architecture of the myocardium. The heart tissues of rats in the ISO group showed evidence of mononuclear infiltration and perforated cardiac myofibers with necrosis. Following the administration of ISO to rats that had previously been treated with aescin at doses of 5, 10, and 20 mg/kg body weight, myocardium of the rats were protected from the damage caused by ISO, as evidenced by a decrease in mononuclear infiltration and a rupture of myofibers with mild to moderate necrosis. 10 mg/kg body weight of aescin exhibited the least amount of mononuclear infiltration and necrosis in the myocardium.

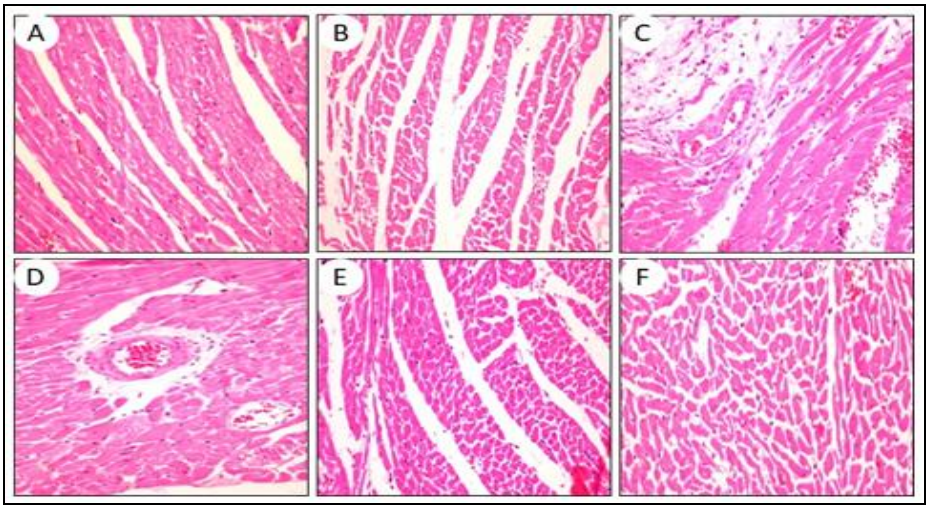

FIG. 7: HISTOPATHOLOGICAL STUDIES OF THE HEART TISSUE OF NORMAL AND EXPERIMENTAL GROUP OF RATS (HEMATOXYLIN AND EOSIN 100X)

DISCUSSION: The animal body weight did not differ significantly across the groups observed in this investigation. The ISO-treated rats exhibited a significantly higher heart/body weight ratio, possibly due to the increased water content, edematous intramuscular space, and ventricular stiffness. The modification in the body and heart weight ratio of the ISO rats was prevented by prior administration of 10 mg/kg b.w of aescin.

In this study, ISO-treated rats showed enhanced activity of hepatic and cardiac marker enzymes (CK, LDH, ALT, CK-MB and AST) in the blood, possibly due to the cardiac membrane damage [29]. Pretreatment with aescin significantly prevented the leakage of all the enzymes from the heart and liver, indicating that it could prevent membrane degradation, thereby inhibiting the leakage of the enzymes. Raafat *et al.* [30] have reported that aescin has vasoprotective and antioxidant effects, which may confer this protective mechanism against oxidative cardiac injury. The most specific markers for MI are serum cardiac troponins (cTns). After myocardial damage due to the increased free radical formation by ISO, cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are released into the bloodstream [31]. In the current work, the level of serum cTnT was elevated in ISO-induced MI rats, and the leakage is a result of heart injury. Aescin-treated ISO group rats showed a normal level of cTnT in the serum. This action is most likely due to the cardioprotective effect of aescin against myocardial damage caused by ISO, which limits the leaking of cTnT from the myocardium into circulation. The results are in agreement with the earlier report [32].

The heart's low-molecular-weight regulatory protein cardiac troponin I (cTnI) regulates the calcium-mediated interactions between myosin and actin [33]. This contractile protein is a sensitive and specific diagnostic marker for MI since it is present only in the heart muscle but is released during MI. In this investigation, rats treated with ISO alone showed a higher level of cTnI when compared to normal rats. These finding is congruent with those of earlier research report [34]. The observed increases in cardiac cTns T and I may be due to the

myocardial injury caused by ISO. Animals pretreated with aescin and then subjected to ISO challenge showed considerably lower serum levels of cTns T and I. This effect might be due to the antioxidant properties of aescin, which may help protect the myocardial membrane's structural and functional integrity, as shown by the absence of cardiac markers in the serum.

Endogenous enzymatic antioxidant defense is essential in preventing tissue damage caused by free radicals. The main free radical scavengers in the first line of cellular defense against oxidative stress are GPx, GST, SOD and CAT, which eliminate hydrogen peroxide (H_2O_2) and oxygen free radicals before they cause oxidative stress [35]. A decrease in SOD activity might lead to a drop in radical anions removal, which could be detrimental to the myocardium. In this study, decreased activities of CAT, SOD, GPx, and GST were observed in the erythrocyte and myocardium of ISO-treated rats. This may be due to the increased generation of lipid peroxides and free radicals [36]. The changes in the activities of enzymatic antioxidants lead to the increased formation of highly potent hydroxyl radicals, superoxide radicals, and H_2O_2. Aescin pretreatment Ameliorates the activities of these enzymatic antioxidants in the erythrocytes and heart tissue, most likely due to its potential to enhance the endogenous antioxidant capacity to scavenge free radicals.

Lipids influences the composition, structure, and stability of the plasma membrane and plays a significant role in the progression of CVD. Meeran *et al.* [37] reported that ISO administration increased the level of lipids in circulation. The current study found that TC, FFA, TGs and PLs were increased in the rats administered with ISO serum, which might be due to enhanced lipolysis in the cardiac membrane. According to Sivasangari *et al.* [38] increased membrane phospholipid breakdown causes cell damage and death. Increased lipolysis and the consequent rise of plasma FFA levels may result in increased hepatic TG production, leading to higher plasma TG and cholesterol concentrations. Increased lipid synthesis through cardiac cyclic adenosine monophosphate (cAMP) may be responsible for the significantly elevated lipid profile reported in rats treated with ISO alone [39]. Pretreatment with aescin prevented the increase of TC, TGs, FFA and PLs in serum on ISO-treated rats. Phospholipase A_2 is known to release free fatty acids from membrane phospholipids through increased peroxidation, and Ca^{2+} ions be one of the phospholipase A_2 activators [40].

Ca^{2+}concentration may increase in the myocardium of ISO rats due to increased cAMP formation by the activation of G-protein coupled receptors by the binding of ISO [41]. ISO increases calcium levels, which might be responsible for the observed increase in FFA concentration. In histopathological analysis, ISO toxicity was evident in the heart tissues of ISO rats, which displayed perforated myofibers with necrosis and fibrosis. The heart tissue from MI rats pretreated with10mg/kg b.w of aescin showed normal architecture of cardiac tissue without necrosis. Aescin treatment protected the myocardium against ISO-induced damage and restored myocardial architecture to near-normal. Histopathological investigations of cardiac tissues from normal and aescin-only treated rats revealed a healthy morphology of the heart muscle without any necrosis, demonstrating that aescin is non-toxic[42]. Aescin 5 and 20 mg/kg b.w also provided excellent morphological protection by reducing muscle fiber loss with moderate necrosis.

The histopathological results commonly supported the biochemical investigations. The antioxidant properties of aescin may contribute to the reported beneficial effects on cardiac tissues.

CONCLUSION: In this study, we observed the increased activity of cardiac markers in the serum of ISO rats, which might be due to the increased level of oxidative damage in the myocardium. ISO-induced ROS production might be the reason for the increased membrane damage in cardiac cells.

The results of our study clearly illustrate that aescin (10 mg/kg b.w) treatment prevented oxidative damage, hyperlipidemia and histopathological alterations induced by ISO, thereby protecting the myocardium from the damage caused by ISO. This cardioprotective nature of aescin might be due to its antioxidant nature.

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CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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