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THE EVALUATION OF CARDIOPROTECTIVE ACTIVITY OF *ECKLONIA CAVA* EXTRACT IN RATS

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ABSTRACT

Acute myocardial infarction is an important ischemic heart disease and a leading cause of morbidity and mortality worldwide. Isoproterenol (ISO) is a synthetic β-adernoceptor agonist that its injection induces myocardial infarction in rats. The goal of this study was to evaluate the cardioprotective effects methanolic extract from a brown algae *Ecklonia cava* (*EC*) against ISO-induced cardiotoxicity in a rat model. A total of 24 rats were divided into four groups: normal, control, low-dose *EC*, high-dose *EC*. Biochemical parameters were measured. The blood was subjected to centrifugation to isolate serum out of it. The serum was further used for estimation of CK-MB, Lactate Dehydrogenase (LDH), Alanine transaminase (SGPT), Aspartate transaminase (SGOT), Total Cholesterol (TC), Triglycerides (TG), LDL, and HDL for Cardioprotective activity. And Antioxidant Enzymes Superoxide dismutase SOD and Choline acetyltransferase CAT of heart tissue were measured to determine the antioxidant activity. Hearts were isolated were observed under light microscope for histopathological study. Methanolic extract of *Ecklonia cava* treated group showed significant reduction in CK-MB, LDH, SGOT, SGPT, TG, TC, LDL and increase in HDL levels when compared to toxic control group, as well as increase in the activities of antiperoxidative enzymes SOD, CAT in the heart tissue and was further confirmed by histopathology study.

KEYWORDS: *Ecklonia cava*, Cardioprotective, Isoproterenol.

INTRODUCTION

Isoproterenol (ISO) is a synthetic β-adernoceptor agonist, myocardial necrosis induced by ISO is probably due to a primary action on the sarcolemma membrane, followed by

stimulation of adenylate cyclase, activation of Ca^{2+} and Na^{+} channels, exaggerated calcium inflow and excess of excitation contraction coupling mechanism leading to energy consumption and cellular death. Free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity.[1]

Brown algae have long been used traditionally as foodstuffs and folk medicines in Korea, Japan, and China. Brown algae have been known to produce many potentially medicinal polysaccharides and lipids such as fucoidan, laminaran, fucoxanthin, and fucosterol. More recently, they have also been shown to produce a special class of polyphenols called ‘‘phlorotannins,’’ which are polymers of phloroglucinol. Among many brown algae, *Ecklonia cava* produces pharmacologically prominent types of phlorotannins called ‘‘eckols.’’ Although *E. cava* also produces other potentially medicinal polysaccharides and lipids as mentioned above, an increasing number of reports indicate that many of the medicinal properties of this brown algae derive from eckols [2][3]. Recently, eckols have been shown to have a broad spectrum of biological activities, including antioxidant activity[4-8] radical scavenging activity[9], matrix metalloproteinase inhibitory activity[10], protease inhibitory activity[11], cytoprotective activity.[12][13] However, its cardioprotective effects remain poorly understood, we have evaluated the cardioprotective effect of *EC* against ISO-induced cardiomyopathy using a rat model.

MATERIALS AND METHODS

A methanolic extract from a brown algae *EC* was kindly supplied by Karnataka College of Pharmacy. The dried powder was extracted three times with ten volumes of methanol at room temperature. Extract was filtered with Whatman filter paper and left to dry for 7 days.

Animals

Albino Wistar rats (n = 24) weighing between 150-200 gm were maintained in standard laboratory conditions at room temperature (25±2°C) with 12 hr. light/dark cycle. The animals were given pellet chow and water ad libitum except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) at Karnataka College of Pharmacy, Bangalore. Studies were performed in accordance with the CPCSEA guidelines.

Experimental protocol: As illustrated in Table 1, rats were divided into four groups each consists of 6 animals: normal, control, low-dose *EC*, high-dose *EC*. The control, low-dose and high dose groups were administrated with 2.4 mg/ kg of ISO) intravenous on 13th and 14th days.[14] On the 15th day blood was collected from the retro orbital route under Ether anesthesia for biochemical estimations. Then all the animals were sacrificed and the parameters was studied are general appearance, gross morphology, biochemical parameters antioxidant activity and histopathology.

Table 1: Experimental protocol.

Group-1	**Normal Group**	Rats were given olive oil orally for 14 days
Group-2	**Control Group**	Rats were given olive oil orally for 14 days + Isoproterenol (2.4 mg/kg I.V) (two consecutive doses on 13th and 14th day)
Group-3	**Low Dose *EC***	Rats were treated with *Ecklonia cava* methanolic extract dissolved in olive oil as solvent orally, low dose (200 mg/kg for 14 days) + Isoproterenol (2.4 mg/kg I.V) (two consecutive doses on 13th and 14th day).
Group-4	**High Dose *EC***	Rats were treated with *Ecklonia cava* methanolic extract dissolved in olive oil as solvent orally, high dose (400 mg/kg for 14 days) + Isoproterenol (2.4 mg/kg I.V) (two consecutive doses on 13th and 14th day).

At the end of the study biochemical parameters were measured. The blood was subjected to centrifugation to isolate serum out of it. The serum was further used for estimation of CK-MB, Lactate Dehydrogenase (LDH), Alanine transaminase (SGPT) and Aspartate transaminase (SGOT) Total Cholesterol (TC), Triglycerides (TG), LDL, and HDL for Cardioprotective activity. The hearts were isolated and examined general appearance, gross morphology, then washed with ice cold saline. The tissue was fixed in 10% buffered formalin neutral solution. After fixation tissue was embedded in paraffin-wax and sections are cut and stained with hematoxylin and eosin. The slides were observed under light microscope.

Statistical Analysis: Results were expressed as the Mean ±standard error means S.E.M.). The comparison of data within groups was performed by the analysis of variance using ANOVA test. Significant difference between control and experimental groups was assessed by Dunnett's test. A probability level of less than 1% ($P < 0.001$) was considered significant. Statistical analysis was performed using Graph Pad prism.

RESULTS

General Appearance and Gross Morphology: *Ecklonia cava* treated rat showed near normal morphology as comparable with that of normal rats. While rats administered with Isoproterenol showed infarct like lesion and morphological alterations in the heart resembling heart failure.

Biochemical Parameters

Rats administered with *Ecklonia cava* methanolic extract given in dose of 200 mg/kg as Low Dose showed a decrease in the levels of serum triglycerides, cholesterol, and LDL and increase in HDL when compared with their respective control rats. Extract given in dose of 400 mg/kg as High Dose showed also similar decrease in the levels of serum triglycerides, cholesterol, and LDL and increase in HDL when compared with their respective control rats.

Table 2: Level of cardiac CK-MB, LDH, SGOT and SGPT in normal and experimental groups of rats.

Groups	CK-MB(IU/L)	LDH (IU/L)	SGOT(IU/L)	SGPT(IU/L)
Normal	48.2411 ± 0.8212	76.6602± 0.5315	43.1219± 0.7065	24.50139± 0.2691
Control (ISO, 2.4 mg/kg)	149.6624± 0.8096$^{\#}$	153.9096± 0.3182$^{\#}$	141.9981± 0.8635$^{\#}$	45.0131± 0.2019$^{\#}$
***EC* Extract (200 mg/kg) + ISO (2.4 mg/kg)**	103.8839± 0.7548**	105.4782± 0.5833**	82.94339± 0.7482**	34.4373± 0.2938**
***EC* Extract (400 mg/kg) + ISO (2.4 mg/kg)**	87.6141± 0.7158**	91.3301± 0.6313**	69.7731± 0.6983**	28.9358± 0.3142**

Values are expressed as Mean ±SEM and n=6, *P<0.01, **P<0.01 using one-way ANOVA coupled with Dunett *t* test **P<0.01 is considered as significant. $^{\#}$ Indicate control group with normal ($^{\#}$P<0.01) and * indicate other group compared with control group.

Figure 1: Effect of *Ecklonia cava* on CK-MB level in rat by ISO induce cardiac toxicity

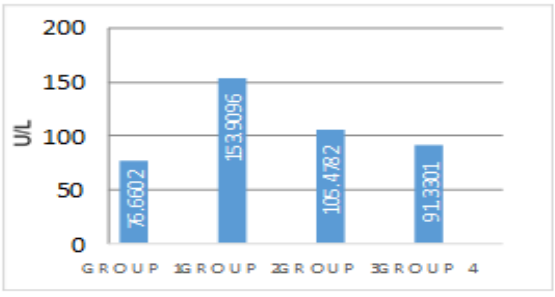


Figure 2: Effect of *Ecklonia cava* on LDH level in rat by ISO induced car

Figure 3: Effect of *Ecklonia cava* on SGPT level in rat by ISO induced cardiac toxicity

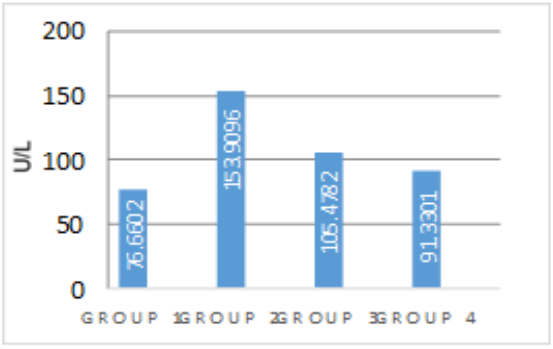


Figure 4: Effect of *Ecklonia cava* on SGOT level in rat by ISO induced cardiac toxicity

Table 3: Effect of *Ecklonia cava* extract on lipid profile in rats.

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal	46.91 ± 3.08	66.13 ± 3.0	18.27 ± 2.81	38.48 ± 0.87
Control (ISO, 2.4 mg/kg)	93.82 ± 5.1$^{\#}$	96 ± 3.3$^{\#}$	60.18 ± 3.6$^{\#}$	17.04 ± 0.6$^{\#}$
Low Dose of *EC* (200 mg/kg)	82.72 ± 3.7*	85.6 ± 5.1*	51.62 ± 4.7*	17.5 ± 0.6
High Dose of *EC* (400 mg/kg)	57.38 ± 2.9*	66.7 ± 2.1*	31.02 ± 1.4*	24.19 ± 0.7*

Values are expressed as Mean ±SEM and n=6, *P<0.01, **P<0.01 using one-way ANOVA coupled with Dunett *t* test **P<0.01 is considered as significant. $^{\#}$ Indicate control group with normal ($^{\#}$P<0.01) and * indicate other group compared with control group.

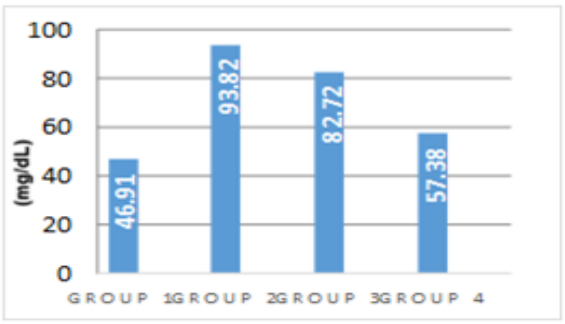


Figure 5: Effect of *Ecklonia cava* on TG level TC level in rat by ISO induced cardiac toxicity toxicity

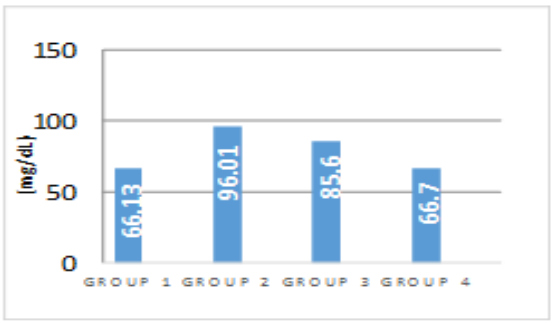


Figure 6 Effect of *Ecklonia cava* on in rat by ISO induced cardiac

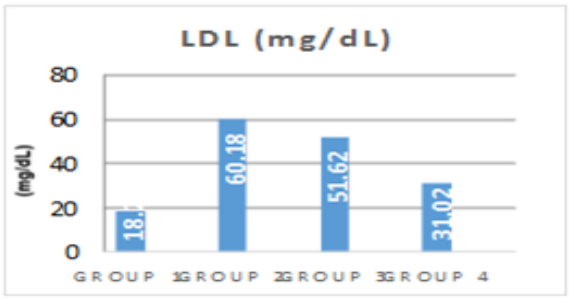


Figure 7: Effect of *Ecklonia cava* on LDL level in rat by ISO induced cardiac toxicity

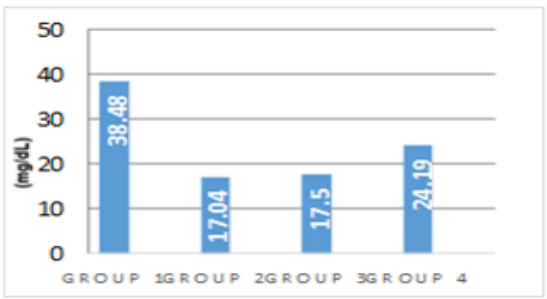


Figure 8: Effect of *Ecklonia cava* on HDL level in rat by ISO induced cardiac toxicity

Antioxidant Activity

Table 4: Antioxidant enzymes of heart tissue homogenate of rats

Group	SOD (IU/mg)	CAT (IU/mg)
Normal	2.2930± 0.0912	35.1814± 0.4013
ISO (2.4 mg/kg I.V.)	0.7325± 0.0661$^{\#}$	16.0212± 0.3306$^{\#}$
Ecklonia cava (200 mg/kg) +ISO (2.4 mg/kg)	1.928± 0.057**	22.3998± 0.2618**
Ecklonia cava (400 mg/kg) +ISO (2.4 mg/kg)	2.0317± 0.0568**	30.2167± 0.2943**

Values are expressed as Mean ±SEM and n=6, *P<0.01, **P<0.01 using one-way ANOVA coupled with Dunett *t* test **P<0.01 is considered as significant. $^{\#}$ Indicate control group with normal ($^{\#}$P<0.01) and * indicate other group compared with control group.

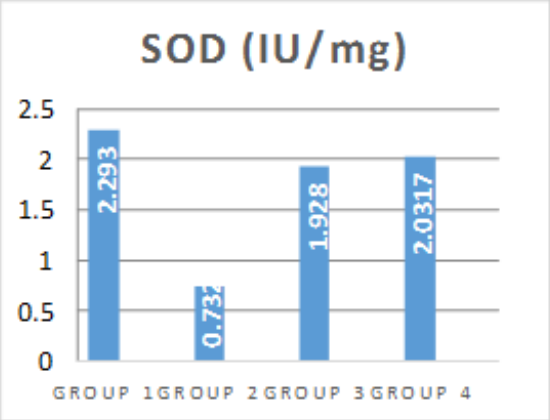


Figure 9: Effect of *Ecklonia cava* on SOD levels in rat by ISO induced cardiac toxicity

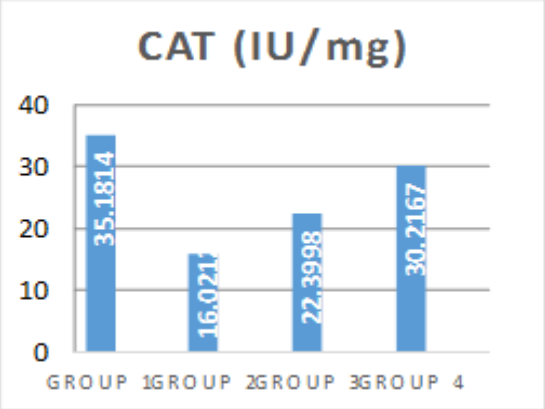
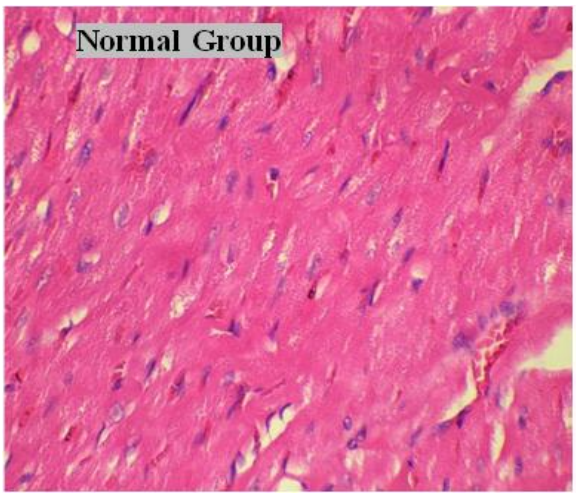
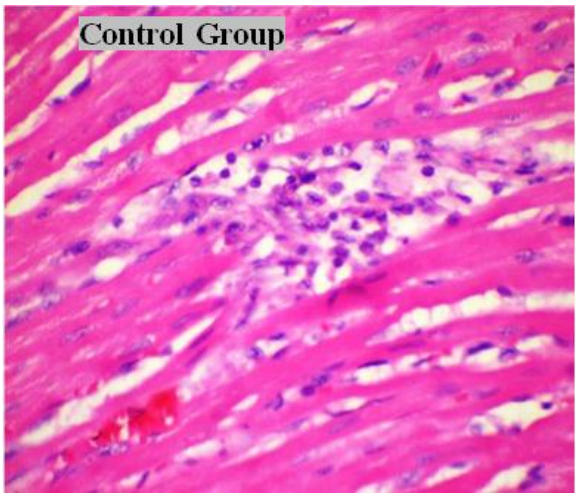


Figure 10: Effect of *Ecklonia cava* on HDL levels in rat by ISO induced cardiac toxicity

Histopathological Findings





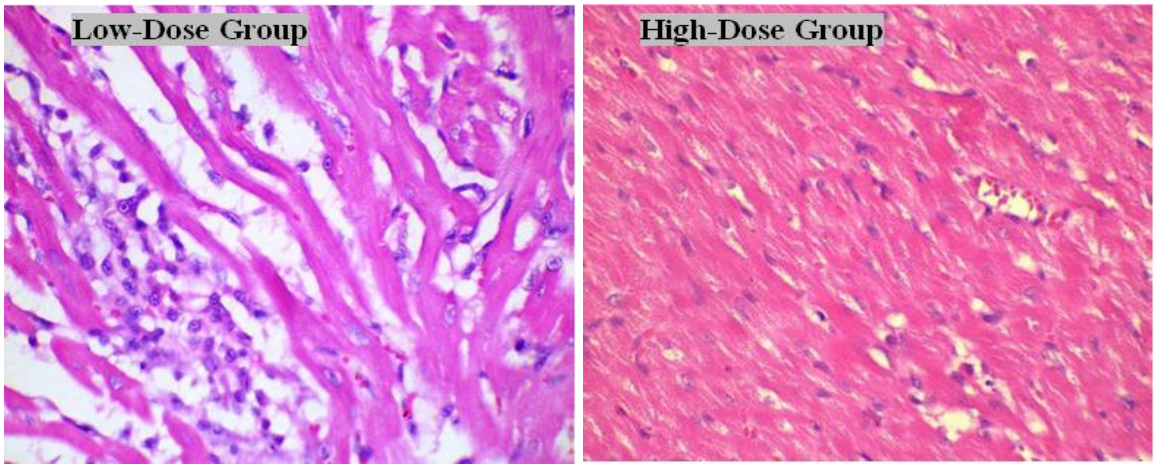


Figure 11: The comparisons of histopathological results. Haphazard arrangement of the cardiac muscle fibers. The cardiac muscle fibers show necrosis consisting of loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent myofibrils were observed in Control group. Considerable depletion of myofibrillar bundles and myofibrillar lysis in Low-Dose group, however, in High-Dose group less myofibrillar damage and rarefaction than in ISO group, intact arrangement of the cardiac muscle fibers. The cardiac muscle fibers show intact integrity of myocardial cell membrane, myofibrillar structure with striations and continuity with adjacent myofibrils. The interstitial space appears mildly increased at focal areas.

Table 5: Tissue damage score in studying groups.

S. No.	Groups	Score	Histopathology findings
1.	Normal Control	0	Normal histology of cardiac myocytes/ NAD
2.	ISO Control	4	Necrotic myofiber areas (3-4) with infiltration of inflammatory cells (1-2) and vacuolar cytoplasmic changes (1-2)
4.	Low dose *EC* (200 mg/kg) +ISO (2.4 mg/kg)	3	Necrotic myofiber areas (2-3) with infiltration of inflammatory cells (1-2) and vacuolar cytoplasmic changes (1).
5.	High dose *EC* (400 mg/kg) +ISO (2.4 mg/kg)	1	Necrotic myofiber areas (1) with infiltration of inflammatory cells (1) and vacuolar cytoplasmic changes (1).

Score 0: Normal arrangement of cardiac fibers, no damage.

Score 1 (mild): Normal arrangement of cardiac fibers, inflammatory infiltrate and focal necrosis.

Score 2 (moderate): Normal arrangement of cardiac fibers, moderate inflammatory infiltrate, diffuse myocardial cell swelling and necrosis.

Score 3 (Severe): Haphazard arrangement of cardiac fibers, Severe inflammatory infiltrate and Necrosis with the presence of contraction bands / Apoptosis /Degenerative cardiocytes. **Score 4 (Highly Severe):** Haphazard arrangement of cardiac fibers, Severe inflammatory infiltrate, Widespread necrosis with presence of contraction bands /Apoptosis/ Degenerative cardiocytes and hemorrhage.

DISCUSSION

Myocardial Ischemia occurs because of atherosclerosis. On the eruption of the plaque reperfusion occurs. During reperfusion free radical generates which causes further damage which may lead to death.[15] In ISO-treated myocardium reactive oxygen species (ROS) are formed at an accelerated rate. Cardiac myocytes, endothelial cells, and infiltrating neutrophils contribute to this ROS production and can lead to cellular dysfunction and necrosis. 'Infarct-like' lesions are produced in the myocardium when injected with ISO. Myocardial necrosis induced by ISO is probably due to a primary action on the sarcolemma membrane, followed by stimulation of adenylate cyclase, activation of Ca2+ and Na+ channels, exaggerated calcium inflow and excess of excitation contraction coupling mechanism leading to energy consumption and cellular death. Free radicals generated by ISO, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity.[1]

Ecklonia cava treated rat showed near normal morphology as comparable with that of normal rats. While rats administered with Isoproterenol showed infarct like lesion and morphological alterations in the heart resembling heart failure. Methanolic extract of *Ecklonia cava* was used to study the Cardioprotective activity. Cardiac enzymes (CK-MB, LDH, SGOT and SGPT) were measured to determine the myocardial damage, Lipid profile (TG, TC, LDL, HDL), as well as SOD and CAT for Antioxidant Activity. The metabolic damage of myocardium results in increase in the concentration of the marker enzymes like CK-MB, LDH, SGOT and SGPT as well as increase in Total Cholesterol, Triglycerides, LDL plasma level with reduction in HDL levels.

Methanolic extract of *Ecklonia cava* 400 mg/kg prevented the alterations in marker enzymes of myocardial infarction when compared with control group and showed significant decrease CK-MB (87.6141± 0.7158**), LDH (91.3301± 0.6313**), SGOT (69.7731± 00.6983**), SGPT (28.9358± 0.3142**). Pre-treatment of methanolic extract of *Ecklonia cava* 200 mg/kg

showed significant decrease in levels of CK-MB (103.8839± 0.7548**), LDH (105.4782± 0.5833**), SGOT (82.94339± 0.7482**), SGPT (34.4373± 0.2938**).

ISO and pre-treatment of methanolic extract of *Ecklonia cava* 400 mg/kg showed significant decrease in the levels of TG (57.38 ± 2.9*), TC (66.7 ± 2.1*), LDL (31.02 ± 1.4*) as well as significant increase in HDL levels (24.19 ± 0.7*), while methanolic extract of *Ecklonia cava* 200 mg/kg showed significant decrease in TG (82.72 ± 3.7*), TC (85.6 ± 5.1*), LDL (51.62 ± 4.7*) and increase in HDL levels (17.5 ± 0.6) when compared to toxic control group. Oral administration of *Ecklonia cava* which contains phlorotannins resulted in hypolipidemic effects in rats. The antidyslipidemic mechanism of the phlorotannins is may be by suppressing the accumulation of fat or promote its metabolism. Based on a recent studies a compound isolated from *EC* inhibited lipid accumulation and adipocyte differentiation with down regulation of the genes involved in lipogenesis and adipogenesis. It is speculated that *EC* contributed to the body fat reducing effect by suppression of adipogenesis and lipogenesis.[16]

Further it is observed that significant increase in the activities of antiperoxidative enzymes (SOD 2.0317± 0.0568**), CAT (30.2167± 0.2943**) in the heart tissue in *Ecklonia cava* phlorotannins -supplemented groups (400 mg/kg).

Rats pre-treated with methanolic extract of *Ecklonia cava* in High Dose (400 mg/kg) showed normal myofibrillar structures with striations (Score: 1) and revealed a marked protection by the extract against myocardial necrotic damage. Low dose (200 mg/kg) showed intact integrity of myocardial cell membrane in cardiac muscle fibers, myofibrillar structure with striations and continuity with adjacent myofibrils. The interstitial space appears mildly increased at focal areas. There are seen few vascular spaces amidst these cardiac muscle fibers, Score (3).

Phlorotannin components of *Ecklonia cava* are potent antioxidants and showed a cytoprotective effect against oxidative stress-induced cell damages in various cell systems through apparently several pathways, including DNA protection, upregulation of intrinsic antioxidation factors, and alleviation of mitochondrial dysfunction.[17] Also, in a recent study using a confocal fluorescence microscopy, dieckol, a major phlorotannin component of *Ecklonia cava*, has been shown to exclusively localize in Endoplasmic Reticulum (ER) and reduce ER stress in macrophage and neuronal cells. It has been shown that phloroglucinol has

anti-inflammatory and anti-oxidant properties. Both inflammatory cell infiltration and myeloperoxidase (MPO) activation play an important role in myocardial reperfusion injury.[18]

In summary, our rat model study results showed that *EC* was safe and cardioprotective against ISO-induced cardiotoxicity in a dose-dependent manner. Especially, High-Dose which had significant cardioprotective effects against ISO-induced cardiotoxicity in a rat model with the evidence based on biochemical results and histopathological findings.

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