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ORIGINAL RESEARCH ARTICLE

Evaluation of Anti-hypertrophic Potential of *Enicostemma littorale* Blume on Isoproterenol Induced Cardiac Hypertrophy

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Abstract The main objective of this study is to evaluate the anti-hypertrophic potential of the aqueous extract of *Enicostemma littorale* (*E. littorale*) against isoproterenol induced cardiac hypertrophic rat models (male albino Wistar rats) through biochemical investigations. Aqueous extract of *E. littorale* known for various beneficial properties was administered (100 mg/kg, 12 days, oral) to isoproterenol (ISO) induced cardiac hypertrophic rats (low ISO—60 mg/kg, 12 days and high ISO—100 mg/kg, 12 days, subcutaneous) and were compared with group that was treated with the reference drug, Losartan (10 mg kg, administered for 12 days, oral). The anti-hypertrophic effect of *E. littorale* was evaluated by analysing the morphometric indices of the heart, ECG tracings, changes in blood biochemical parameters viz., serum glucose, serum total protein, serum albumin, lipid profile, cardiac specific enzymes (SGOT, SGPT and LDH) and histopathological examination of the heart tissue. The results fundamentally revealed that the plant extract efficiently ameliorated cardiac hypertrophy induced by ISO injected in experimental rats. The outcomes of biochemical investigations of this study highlighted the association between the hypertrophic β-adrenergic receptor signalling (β-AR) and the 5′ AMP-activated protein kinase (AMPK)—peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) axis in the metabolism of cardiac fibrosis and hypertrophy. This β-AR/AMPK-PGC1α signalling stem can serve as a key target in ameliorating cardiac hypertrophy through focus on its principal regulators. To add, we also propose that the glycoside, swertiamarin present in this plant with the reported anti-fibrotic potential in liver can be further isolated and evaluated for its anti-hypertrophic potential to treat cardiac hypertrophy.

Keywords Cardiac hypertrophy · Cardiac fibrosis · Isoproterenol · *Enicostemma littorale* · Swertiamarin · Anti-hypertrophic effect

Introduction

Cardiac hypertrophy (CH) is defined as the adverse remodelling process of the cellular architecture of the heart that arises initially as an adaptive mechanism towards stressful conditions which on prolonged state leads to the critical intermediate stage that advances into different cardiovascular complications due to the various intrinsic, extrinsic, physiological and pathological stimuli [9]. The characteristic features of CH are fibrosis (enhanced sarcomere organization—extracellular matrix deposition) and inflammation accompanied by increase in cardiomyocyte (CM) size and protein synthesis, thickening of left ventricle and a shift in energy fuel substrates (from fatty acid to glucose) [25]. Besides aging, genetic mutations, obesity, reactive oxygen species (ROS), various other pathological conditions such as hypertension, diabetes mellitus, valve disorders, myocardial ischemia and renal inefficacy are the factors that drive CH. This state of CH results in Hypertrophic Cardiomyopathy (HCM) and Dilated Cardiomyopathy that ultimately lead to heart failure or cardiac arrest (sudden death). Hence, a holistic control over CH using effective therapeutic strategies with less or no detrimental effects is essential [36].

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Encisotemma littorale (Indian Gentian) belonging to Gentianaceae family of medicinal plants possess enormous pharmacological activities namely antioxidant, anti-inflammatory, anti-hyperlipidaemic, anti-diuretic, antimicrobial, anti-nociceptive, hepatoprotective properties and thereby was used in the treatment of cancer, diabetes mellitus, adrenaline induced hypertension, major depressive disorder, atherosclerosis and liver fibrosis [8, 12, 28]. Enormous interests are directed at the very recently found potential of one of its unique phytocompound, Swertiamarin (a seco-iridoid glycoside) in treating liver fibrosis by evaluating its possible inhibitory and attenuating potential of Angiotensin II receptor (AT_1R) signalling, transforming growth factor-beta (TGF-β) signalling and Nuclear factor erythroid 2–related factor 2 (NRF2) signalling mechanisms [7, 18, 37].

Isoproterenol, a β-adrenergic receptor (β-AR) agonist causes concomitant activation of β-AR leading to increased TGF-β signalling resulting in CH in rat models. *E. littorale* was reported to possess anti-fibrotic activity by inhibiting TGF-β signalling. Therefore, we hereby hypothesize that *E. littorale* may ameliorate the fibrotic events of TGF-β pathway, thereby may attenuate the progression of CH.

Materials and Methods

Collection of Plant Materials

Encisotemma littorale (whole plant) was collected from the local herbal shops in Coimbatore district, Tamil Nadu, India, and was authenticated by the Botanical Survey of India, Southern Regional Centre, Coimbatore (No. BSI/SRC/5/23/2010-11/Tech-2051).

Preparation of Aqueous Extract of *E. littorale*

The vegetative and reproductive parts of *E. littorale* (150 g) were shade dried, powdered in a mixer grinder, and stored in airtight containers. The dried plant powder was mixed with water (1:4 ratio) [14], cold macerated for 72 h with intermittent shaking, filtered and the filtrate was then concentrated to dryness under reduced pressure at controlled temperature in a water bath [12].

Chemicals

All the chemicals were of analytical grade purchased from Hi-Media Laboratories Pvt., Limited, (Mumbai, Maharashtra, India). Isoproterenol (Isoprenaline hydrochloride), Losartan (Losarpen—25 mg) were purchased from Sigma-Aldrich and local retail pharmacy (Coimbatore), respectively. Glucose and cholesterol estimation kits, Triglyceride, SGOT and SGPT kits were purchased as from, Arkray Healthcare, Ensure Biotech and Microlyn Healthcare (India), respectively.

Experimental Animals

Healthy male albino Wistar rats (8 weeks old weighing 180–200 g) were procured and ethical clearance for handling of these experimental animals was obtained from the Institutional Animal Ethics Committee (IAEC) of PSG Institute of Medical Sciences and Research, Coimbatore, that acts under the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (CPCSEA/No: 386/2018/IAEC). The animals were acclimatized under standard laboratory conditions for 3 days with controlled temperature (29° ± 5 °C), humidity (55% ± 5%), and 12 h of light/dark cycles and maintained under the same conditions throughout the experimental period.

Experimental Groups: Induction and Treatment of CH

The experimental animals were divided into seven groups (6 rats each group) and the doses of compounds were fixed based on previous literatures as shown in Table 1. Blood was collected at regular intervals (every 3 days) by retro-orbital bleeding for biochemical analysis.

Electrocardiography (ECG)

To monitor the cardiac morphological and rhythmic changes in vivo, ECG analysis on unanaesthetised rats with conventional bipolar limb lead II was performed using BITalino ECG Sensor (PLUX—Wireless Biosignals, Lisbon, Portugal) with acquisition by BITalino Opensignals [r]evolution Software using Einthoven triangle configuration (1000 Hz;100 ms and 1 mV) [29]. Recordings were carried out over 6 min to obtain stabilized ECG tracings. Analysis of cardiac function by ECG recordings were performed for all animals to screen for changes in QRS, S–T waves and R–R interval associated with CH.

Collection of Serum and Heart Tissues

After ECG recordings and confirmation of CH the animals were anaesthetized using mild chloroform. Cardiac puncture method was used for collecting the blood, allowed to clot (30 min at room temperature) and the clotted blood was centrifuged at 5000 rpm at 4 °C for 20 min to obtain the serum. The separated sera were stored in small aliquots at − 20 °C and used for analysis to avoid repeated freezing

Table 1 Experimental rat models of cardiac hypertrophy induced by ISO and treated using losartan and aqueous extract of *E. littorale*

Groups	Experimental animals	Experimental set-up
I	Control	Normal
II	ISO low	Isoproterenol (ISO-low) (60 mg/kg b.w., s.c., 12 days)**
III	ISO low + losartan	Isoproterenol (ISO-low) + losartan (10 mg/kg b.w., oral., 12 days)$^{\#}$
IV	ISO low + plant extract	Isoproterenol (ISO-low) + plant extract (100 mg/kg b.w., oral., 12 days)$^{\psi,\varphi}$
V	ISO high	Isoproterenol (ISO-high) (100 mg/kg b.w., s.c., 12 days)†
VI	ISO high + losartan	Isoproterenol (ISO-high) + losartan (10 mg/kg b.w., oral., 12 days)
VII	ISO high + plant extract	Isoproterenol (ISO-high) + plant extract (100 mg/kg b.w., oral., 12 days)

Symbols **, $^{\#}$, $^{\psi}$, $^{\varphi}$ and † point to references 15, 18, 12, 17 and 4, respectively. Dosage fixation, routes and duration of administration of chemicals and plant extracts were based on these references

and thawing. The heart was excised, thoroughly washed in ice-cold saline and used for biochemical and histopathological analysis.

Morphometrics of CH

The degree of cardiac hypertrophy was assessed using the following morphometric (hypertrophic) indices (i.e.) final body weight (BW), Heart weight (HW), HW/BW ratio [25], HW/Tail length (TL) ratio [10].

Biochemical Analysis

The collected sera were used for the estimations of glucose, total protein, albumin, lipid profile (total cholesterol, Triglycerides, HDL cholesterol, LDL cholesterol and VLDL cholesterol) followed by analysis of cardiac markers (SGOT, SGPT and LDH).

Colorimetric determination of serum glucose was assayed using glucose oxidase method (GOD-POD End Point Assay, AUTOSPAN Liquid Gold Glucose Kit), serum total protein by Lowry's method [20], serum albumin using Bromocresol green end point assay method (AUTOSPAN Liquid Gold Albumin), estimation of serum total cholesterol and HDL cholesterol (after separation) using POD-PAP enzymatic end point assay (AUTOSPAN Liquid Gold Cholesterol Kit). Determination of triglyceride was performed using enzymatic method (Triglycerides LS kit), estimations of SGOT and SGPT activities were determined by modified IFCC method (Microlyn Kit) and estimation of LDH activity was done by optimised DGKC, Kinetic assay method (AUTOSPAN Liquid Gold Lactate Dehydrogenase Kit).

Histopathological Analysis

The cleaned excised rat hearts were initially preserved in 10% formalin immediately until tissue processing as transverse, 5-µm-thick paraffin, mid-ventricular sections. These sections were stained with haematoxylin and eosin (H&E) and the cellular architecture of the heart tissues were investigated by scanning the H&E-stained slides (4×) [2].

Statistical Analysis

All the data obtained were expressed as mean ± standard error of the mean (SEM) and statistically verified using Student's *t* test [31] employing SPSS 10.0 software with level of significance set up at $P < 0.05$. The bar graphs were plotted using Microsoft Office Excel 2016.

Results

Yield of Aqueous Plant Extract

The yield of aqueous extract as hygroscopic, brown residue of *E. littorale* was 15 g (30% of the initial amount of plant taken for extraction).

In Vivo Electrocardiographic (ECG) Assessment of the Status of CH

As shown in Fig. 1 and Table 2, the raw ECG wave patterns indicate the occurrence of hypertrophic changes in low ISO (Fig. 1b) and high ISO (Fig. 1e) administered groups with increased QRS interval, increased R–R interval, large QT interval, mildly depressed S–T wave (low dose) and extremely depressed S–T wave (high dose) and reduced heart rate which were abolished in plant extract administered groups (Fig. 1d, g). Similar but lesser restorative effects were seen in losartan administered groups (Fig. 1c, f).

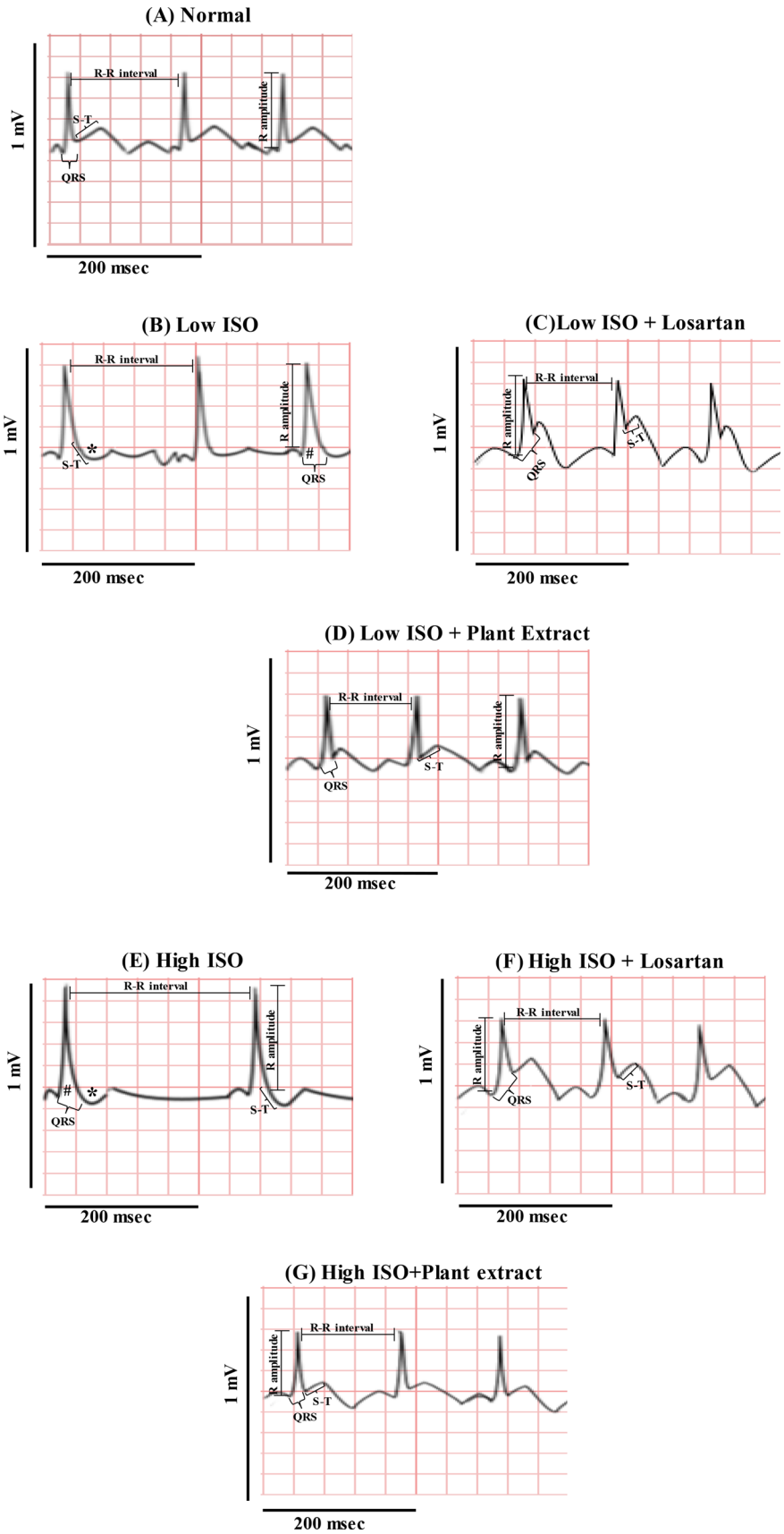

Fig. 1 Graphical representation (to the scale) of the real time ECG tracings (raw) (Lead II) of cardiac assessment using BITALINO Electrocardiography Sensor and Open Signals Software. Increased QRS interval, R–R interval and depressed S–T wave are observed in ISO administered groups (**b**, **e**) as an indication of ventricular dysfunction. All these changes were found to be restored similar to normal during treatment with Losartan (**c**, **f**) and plant extract (**d**, **g**). In this figure * indicates S–T depression and # indicates wider QRS complex

Table 2 ECG parameters of cardiac hypertrophy in experimental groups

Groups	QRS (ms)	S–T (mV)	R amplitude (mV)	R–R interval (ms)	Heart rate (bpm)
Group I—normal	26.10 ± 0.6	0.09 ± 0.01	0.33 ± 0.01	133.03 ± 0.3	450.33 ± 1.5
Group II—low ISO	**40.69 ± 0.4[a]***	**− 0.06 ± 0.01[a]***	**0.56 ± 0.01[a]***	**173.22 ± 0.7[a]***	**346.09 ± 0.7[a]***
Group III—low ISO + drug	40.14 ± 0.5[b]	0.06 ± 0.01[b]*	0.33 ± 0.01[b]*	135.13 ± 0.8[b]*	442.01 ± 0.04[b]*
Group IV—low ISO + plant	33.47 ± 0.5[c]*	0.08 ± 0.01[c]*	0.34 ± 0.01[c]*	134.58 ± 0.5[c]*	446.00 ± 0.09[c]
Group V—high ISO	**41.11 ± 0.5[d]***	**− 0.11 ± 0.03[d]***	**0.52 ± 0.03[d]***	**226.46 ± 0.4[d]***	**264.92 ± 0.9[d]***
Group VI—high ISO + drug	39.97 ± 0.05[e]*	0.11 ± 0.02[e]	0.33 ± 0.01[e]*	134.32 ± 0.3[e]*	436.34 ± 0.4[e]*
Group VII—high ISO + plant	27.69 ± 0.6[f]*	0.09 ± 0.01[f]*	0.33 ± 0.01[f]*	134.73 ± 0.6[f]*	447.49 ± 0.4[f]*

Values in bold fonts denote severe alterations

Table values are expressed by mean ± SD of 6 samples per group. Group comparison: [a]normal (I) versus low ISO (II); [b]low ISO (II) versus Drug (III); [c]low ISO (II) versus herb extract (IV); [d]normal (I) versus high ISO (V); [e]high ISO (V) versus drug (VI); [f]high ISO (V) versus herb extract (VII). Statistical significance is indicated by * symbol

Hypertrophic Parameters

As shown in Table 3, ISO (low and high) groups (II, V) exhibited significantly increased cardiac hypertrophic indices [HW/BW; HW/tail length and Heart index] when compared to normal group. Treatment with losartan and plant extract significantly reduced hypertrophic indices when compared to disease control groups.

Effect of ISO and Plant Extract on Serum Total Protein

Low and high doses of ISO injection to experimental animals significantly increased the serum total protein when compared to the normal group. Low ISO group rats treated with the plant extract showed significant decrease in the raised protein levels whereas the high ISO treated with plant extract was represented with elevated protein levels in comparison to losartan treated groups as shown in Fig. 2.

Effect of ISO and Plant Extract on Serum Albumin

Administration of low and high doses of ISO to experiment groups significantly reduced the serum albumin levels. These levels were found to be significantly raised to near normal values in groups that were followed by simultaneous treatment using plant extract or losartan as given in Fig. 2.

Effect of ISO and Plant Extract on Serum Glucose

Administration of ISO in experimental animals resulted in significant increase in serum glucose levels (low ISO—125.13 ± 0.4 mg/dl; high ISO—143.97 ± 0.8 mg/dl) when compared to the normal group (110.58 ± 0.9 mg/dl). It is to be highlighted that the treatment using plant extract to both low and high ISO administered groups had significantly brought down the increased glucose levels (111.89 ± 1.3 mg/dl and 110.83 ± 1.7 mg/dl, respectively) near to normal levels as similar as that of losartan

Table 3 Primary cardiac hypertrophic indices

Groups	Body weight (g)	Heart weight (mg)	HW/BW	Heart weight/tail length	Heart index [(HW/BW) × 100]
Group I—normal	150.33 ± 2.52	320.56 ± 2.79	2.12 ± 0.02	16.87 ± 0.147	212.14 ± 2.528
Group II–low ISO	180.33 ± 5.51[a]	600.76 ± 1.1[a]*	3.32 ± 0.2[a]*	35.34 ± 0.01[a]*	332.24 ± 10.80[a]*
Group III—low ISO + drug	160.66 ± 4.04[b]*	470.1 ± 3.65[b]*	2.91 ± 0.1[b]*	27.42 ± 0.20[b]	291.50 ± 9.616[b]*
Group IV—low ISO + plant	180.33 ± 3.51[c]*	479.2 ± 0.70[c]*	2.67 ± 0.1[c]*	26.11 ± 0.04[c]*	267.32 ± 6.011[c]*
Group V—high ISO	250.33 ± 3.51[d]*	848.3 ± 2.21[d]*	3.38 ± 0.1[d]*	39.52 ± 0.12[d]*	338.76 ± 3.314[d]*
Group VI—high ISO + drug	180.33 ± 5.51[e]*	588.71 ± 1.2[e]*	3.26 ± 0.2[e]*	36.51 ± 0.07[e]*	326.94 ± 11.35[e]*
Group VII—high ISO + plant	210.66 ± 6.1[f]*	646.41 ± 5.0[f]*	3.06 ± 0.1[f]*	31.03 ± 0.26[f]*	306.53 ± 8.696[f]*

Table values are expressed by mean ± SD of 6 samples per group. Group comparison: [a]normal (I) versus low ISO (II); [b]low ISO (II) versus drug (III); [c]low ISO (II) versus herb extract (IV); [d]normal (I) versus high ISO (V); [e]high ISO (V) versus drug (VI); [f]high ISO (V) versus herb extract (VII). Statistical significance is indicated by * symbol

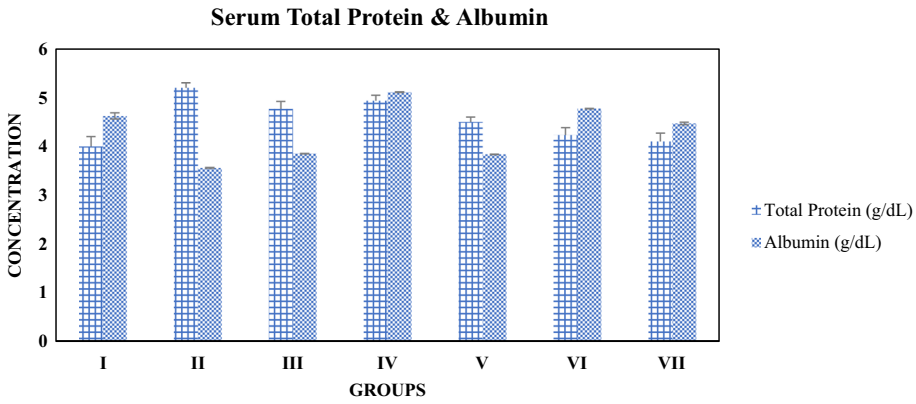

Fig. 2 Effect of ISO and plant extract on serum total protein and serum albumin. Increased serum total protein and decreased serum albumin levels were observed in ISO administered groups (II and V). These changes were reversed when treated with plant extract (IV and VII) on par with the losartan administered groups (III and VI)

administered groups (111 ± 0.6 mg/dl and 129.72 ± 0.9 mg/dl).

Effect of ISO and Plant Extract on Serum Lipid Profile

Lipid parameters namely serum total cholesterol, LDL and VLDL cholesterol showed significant increase in ISO (low and high doses) induced CH groups which were then found to be more significantly reduced to normal level upon treatment with plant extract than with losartan treatment. Triglycerides and HDL cholesterol levels were reduced significantly during diseased state when compared to normal groups which were then restored to near normal levels significantly when treated with losartan and plant extract of which latter showing better effect as shown in Fig. 3.

Effect of ISO and Plant Extract on Cardiac Enzymes

The cardiac enzymes SGOT and SGPT activities were significantly high in both doses of ISO administered groups. Losartan and plant extract administered groups reverted the enzyme activities of which the plant extract showed more effective recovery. With respect to LDH, its levels were low in both the ISO administered control groups whereas its levels were near to normal in ISO groups that were treated with plant extract and losartan as indicated in Fig. 4.

Histopathological Studies

Microscopic examination of H&E stained heart tissues from ISO administered rats revealed separated cardiac myofibrils, lack of intact cardiomyocyte array represented by distorted striations (Fig. 5b, e). Treatment with losartan (Fig. 5c, f) revealed improved organized myofibrils and the administration of plant extract resulted in renewed and rejuvenated architecture (Fig. 5d, g) similar to the normal cardiac tissue with nucleated intact striations (Fig. 5a).

Discussion

Chronic administration of isoproterenol (ISO) administration is the widely used method for inducing cardiac hypertrophy in animal model because of its constitutive stimulation of β-adrenergic (β-AR) signalling associated ROS generation, oxidative stress and altered cellular survival cascades which overall aid in the development of cardiac hypertrophy (CH) in rats [38]. Though *E. littorale* and its major phytoconstituent (swertiamarin) are reported for their potential to ameliorate the factors such as diabetic

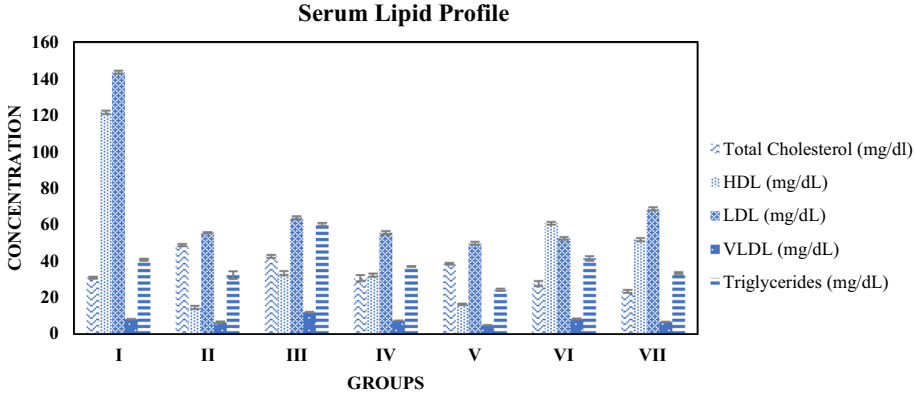

Fig. 3 Effect of ISO and plant extract on serum lipid profile. The increased serum total cholesterol levels accompanied by reduced serum HDL, Triglycerides, LDL and VLDL levels were observed in ISO groups (II and V) which were recovered on treatment with plant extract (IV and VII) and losartan administered groups (III and VI)

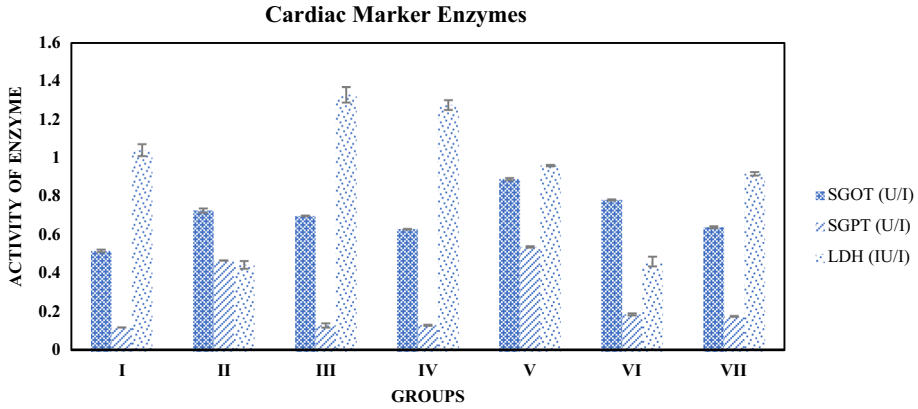

Fig. 4 Effect of ISO and plant extract on cardiac enzymes. Elevated SGOT and SGPT and decreased LDH levels were observed in ISO administered groups (II and V) which were then reverted to near normal levels more efficiently when treated with plant extract (IV and VII) than losartan administered (III and VI) rats

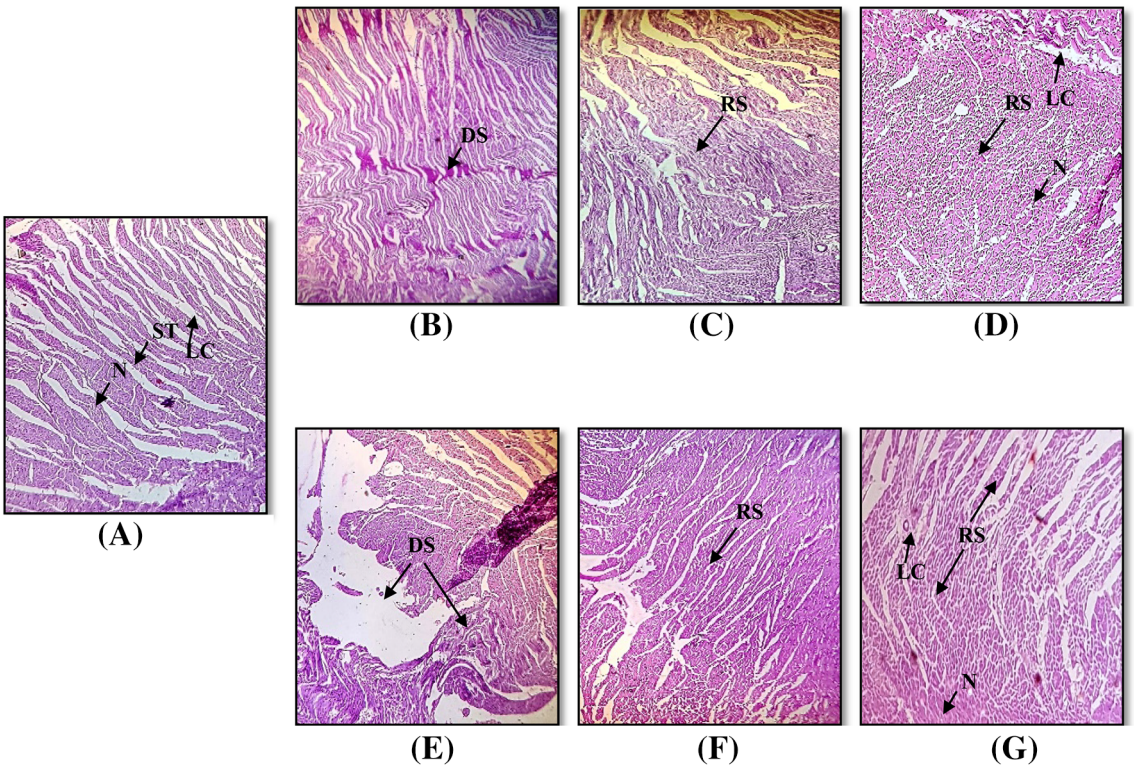

Fig. 5 Histopathological investigation of heart tissues treated with plant extract and losartan to ISO administered rats. Results of H&E stained and scanned (×4) of cardiac muscle (myofibrils) indicated disorganised cardiomyocyte architecture (as curves of damaged striations) in untreated ISO group as result of myocyte necrosis, apoptosis, impaired cell communication that leads to inflammation (due to ROS) followed by interstitial fibrosis (**b**, **e**) when compared to normal that contains typical intact striations with nuclei (as dots) (**a**). Repairment of cardiac myofibril arrangement can be visualized in losartan administered ISO groups (**c**, **f**) and plant extract treated groups (**d**, **g**). *ST* indicates striations, *N* indicates nuclei, *DST* is distorted striations, *RST* means restoration of striations, *LCT* is loose connective tissues

complications, hypertension, oxidative stress that lead to CH, yet their direct therapeutic action on CH has not been investigated so far.

Electrocardiography (ECG) has been used as a screening method for cardiac function in therapeutic studies namely, compounds that inhibit TGF-β signalling [35] in preventing heart complications. In this study, as shown in Fig. 1, the ECG tracings with changes in typical wave patterns were recognized, namely the prolonged QRS, ST wave depression, larger PR interval (LPR) accompanied by larger QT (LQT) interval and increased R–R interval (IRR) (Fig. 1b, c). These changes are indications of impaired atrioventricular conduction, prolonged ventricular repolarization and arrhythmias marked by reduced heart rate (bpm) which were produced due to myocardial fibrosis that may be the cause of ischemia [22, 30]. These alterations were reduced

in rats treated with losartan (Fig. 1d, f) and it is to be highlighted that treatment using plant extract abolished all these typical characteristic ECG changes (Fig. 1d, g) indicating the cardioprotective activity of the plant extract towards restoring normal conduction system of the heart.

The morphometric (hypertrophic) indices being the primary indicators of CH phenotype that represents myocardial fibrosis along with net increase in protein biosynthesis [7, 19]. These histological markers of CH are attributed to the adverse oedematous intramuscular space and necrosis of cardiac myofibrils followed by invasion of damaged tissues by inflammatory cells which are ameliorated or restored to normal state after the treatment [2]. Compared to normal, herein, the ISO administered rats were indicated by increased heart index (HW, BW ratios—64 to 70%) and HW/tail length ratio (42–47%) which overall were reduced by 70–80% and 60–77% of HW/BW and HW/tail length ratios respectively, when treated with plant extract.

Constitutive effects of ISO led to the inhibition of insulin secretion [40] resulting in hyperglycaemia as shown in this study that further imposes energy burden to the cardiac system [6] thereby aggravating the cause of inducing CH by promoting structural and functional changes in cardiac myocytes which were found to be ameliorated by the anti-hyperglycaemic potential of the plant extract.

This study revealed increased serum total protein level in ISO administered rats similar to its chronic administration (30 mμg/100 g body weight, 21 days) in animal models reported increased rates of protein synthesis and hence, the elevated total protein content of the heart during CH [11]. This condition was found to be either decreased or reverted to normal levels in plant extract administered rats which thereby indicates the resilient potential of the plant extract in regulating protein synthesis/expression in cardiac hypertrophy as witnessed in clinical conditions namely cancer, diabetes, depression, obesity and liver disorders [12, 18, 21, 34].

In this study, the lowered levels of serum albumin (hypoalbuminemia) in ISO group can be related to the report that ISO is the strongest binding partner to serum albumin (non-saturable manner) [27] thereby producing adverse myocardial damage due to the reduced antioxidant potential of albumin [19] and hence considered as a high-risk factor of CH, particularly in, left ventricular hypertrophy [23]. Restoration from hypoalbuminemia observed in *E. littorale* extract can be associated to its inhibition on the denaturation of serum albumin in a dose-dependent manner in studies of ulcer and inflammation [26].

Research studies have shown that ISO administration increased the AMPK activity that concordantly enhanced fatty acid oxidation via impaired expression of PPARs thereby shifting the homeostasis towards lipolysis than lipogenesis [1, 3, 32] reflected by increased total cholesterol and the reduced HDL cholesterol, LDL cholesterol, VLDL cholesterol and triglycerides levels as seen in this study. Aqueous extract of *E. littorale* and its phytocompound, swertiamarin were reported to correct the impaired overall lipid metabolism through the regulation of PPAR-γ expression and HMG-CoA reductase activities [24] hence explaining the reparative effect of plant extract on the lipid metabolism.

As chronic ISO administration leads to cellular stress and necrosis of myocytes and other organs, enhanced serum SGOT [34] and SGPT seen in this study could be attributed to the effect of ISO. LDH another cardiac enzyme marker was reported to be transcriptionally reduced by negative regulation of PGC1α, the key regulator of mitochondria was reported to increase in activity with the increase in AMPK levels [5, 33]. In this study, ISO administration could have increased AMPK activity which in turn could have caused reduced LDH activity and their restoration to normal levels by *E. littorale* treatment is suggestive of the reduction in the AMPK activity/expression that demands molecular level investigation of AMPK signalling mechanism on the effect of plant extract or swertiamarin.

Histologically, myocardial degeneration accompanied by myocyte necrosis, apoptosis and altered cell membranes were observed in ISO administered rats probably as a result of the altered glucose and lipid metabolisms. This leads to elevated ROS which activates the signalling networks associated with the deposition of extra-cellular matrix protein (preferably collagen) resulting in inflammation and interstitial fibrogenesis [16, 38, 39]. This remodelling was found to be recovered by the antioxidant potential and metabolism regulating activities of the plant extract which plays major factor in the prevention of myocardial oxidative damage.

Recently, Swertiamarin was widely reported as an effective compound treating hepatic fibrosis through alleviation of oxidative stress and cellular fibrosis via possible cellular signalling pathways that are still under exploration [7, 18, 37]. To add, *E. littorale* and swertiamarin stated to treat diabetic induced renal hypertrophy by ameliorating glomerulosclerosis and interstitial fibrosis are still under investigations [32] and is suggested as promising therapy for diabetic cardiomyopathy (characterised by cardiac fibrosis/hypertrophy during hyperglycaemia) [13].

Conclusion

Hence, the biochemical, physiological and histopathological investigations of the present study strongly established the cardioprotective activity, in particular, the first report

on the anti-hypertrophic potential of the aqueous extract of *E. littorale* represented by its regulatory effect on the glucose, protein and lipid systems which are the key fundamental factors for normal cardiac metabolism.

Future Perspectives

This study envisages intense investigations in future on the remodelled β-adrenergic–PGC 1α–AMPK-metabolic signalling that leads to cardiac fibrosis which can unravel many therapeutic targets and treatment options in cardiac hypertrophy. Therapeutic interventions of *E. littorale* extract or swertiamarin as a principal therapeutic agent responsible for ameliorating and reverting cardiac hypertrophy can be investigated.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

Ethical Approval This article does not contain any studies involving human participants performed by any of the authors. The study was approved by the PSG Institutional Animal Ethics Committee (PSG Institute of Medical Sciences and Research), Coimbatore, India and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India for providing animal ethical clearance (CPCSEA/No: 386/2018/IAEC).

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