International Journal of Drug Development and Research

Monisha et al., Int J Drug Dev & Res 2017, 9:2

Research Article

Preparation of Poly Herbal Formulation (PHF) Extracts and Effect of Histopathological and TCA Cycle Enzymes on Isoproterenol Induced Mycocardial Rats

Sudhakar Monisha[1]*, Marimuthu Ramar[2]*, Balliah Ragavan[1], Pandian Manonmani[3] and Ponnan Arumugam[2]

[1]*Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India*
[2]*Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India*
[3]*Department of Biotechnology, Centre Research and Development, PRIST University, Vallam, Tamil Nadu, India*

***Corresponding author:** Sudhakar Monisha, Department of Biochemistry, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India, Tel: 04224303300; E-mail: moni1487@gmail.com

Marimuthu Ramar, Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu-641 046, India, Tel: 9500336440; E-mail: ramareri@gmail.com

Received March 17, 2017; **Accepted** April 28, 2017; **Published** May 02, 2017

Abstract

The present study was designed to evaluate the cardioprotective role of Poly herbal formulation (PHF) extract on isoproterenol induced myocardial infarction in Wistar albino rats. The rats were divided into eight groups of six animals each. Group I served as a normal control, Group II rats were administered isoproterenol (20 mg/g B.wt). Group III and IV were pretreated with PH extract (250 mg/kg B.wt, 500 mg/kg B.wt, respectively) and received a subcutaneous injection of isoproterenol (20 mg/kg, B.wt) at the end of the experimental period for 2 consecutive days. Group V and VI were pretreated orally with Propranolol (10 mg/kg B.wt, 20 mg/kg B.wt) and received a subcutaneous injection of isoproterenol (20 mg/kg, B.wt) at the end of the experimental period of 2 consecutive days. Group VII and VIII received PH extract of 250 mg/kg B.wt and 500 mg/kg B.wt for 30 days and were studied on the enzymes of TCA cycle, such as isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, and α-ketoglutarate dehydrogenase and respiratory chain enzymes such as NADH dehydrogenase along with histopathological observation. Isoproterenol induction also showed significant ($p<0.05$) decrease in the activities of mitochondrial TCA cycle enzymes such as isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase, and α-ketoglutarate dehydrogenase and respiratory chain enzymes such as NADH dehydrogenase. The PH crude extract produced a significant ($p<0.05$) increase in the TCA cycle enzymes in heart tissue homogenate. The PH crude extract showed a significant ($p<0.05$) improvement in the treated groups. The effect of oral administration of the PH crude extract at the dose of 500 mg/kg B.wt was more than 250 mg/kg B.wt. Similarly the standard drug propranolol treated group 20 mg/kg B.wt also showed a better result. As further confirmed histopathologically, our findings strongly suggest that the cardioprotective effect of the PH crude extract on the myocardium. This result indicates that the PH crude extract exhibit the cardioprotective activity and the protective effect could attribute to its TCA cycle enzyme action.

Keywords: Poly herbal formulation; Crude extracts; TCA cycle enzymes; Histopathological; CVD; Myocardial rats

Introduction

Cardiovascular disease (CVD) is the leading cause of death in most of the developing countries like India. The herbal medicine usage has been increasing over the past decade to cure some of the disorders [1]. Epidemiologists in India and international agencies such as the World Health Organization (WHO) have been sounding an alarm on the rapidly rising burden of CVD for the past 15 years. The reported prevalence of Coronary Heart Disease (CHD) in adult surveys has risen fourfold in 40 years and even in rural areas the prevalence has doubled over the past 30 years. It is estimated that by 2020, CVD will be the largest cause of disability and death in India [2]. CVD has become a universal cause of morbidity and a leading contributor to mortality in both developed and developing countries [3]. Myocardial Infarction or acute myocardial infarction (AMI) is the medical term for an event commonly known as a heart attack. It happens when blood stops flowing properly to part of the heart and the heart muscle is injured due to not receiving enough oxygen. Usually this is because one of the coronary arteries that supplies blood to the heart develops a blockage due to an unstable buildup of white blood cells, cholesterol and fat [4,5].

Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy [6]. Addition to supplying cellular energy, mitochondria are involved in other tasks such as signaling, cellular differentiation, cell death, as well as maintaining the control of the cell cycle and cell growth [7]. Mitochondria are generally considered as origin, but also as a target for reactive oxygen species [8]. They are continuously exposed to a flux of reactive oxygen species (ROS) either produced by respiratory complexes or by other sources such as microsomal oxygenases and extracellular inflammatory responses [9].

Isoproterenol (1- [3,4 dihydroxy phenyl]-2-isopropyl amino ethanol hydrochloride) is a synthetic catecholamine and β-adrenergic agonist, which has been found to cause severe stress in the myocardium resulting in infarct like necrosis of heart muscles [10]. During this reaction, highly toxic oxygen derived free radicals are generated, which are detrimental to extracellular and intracellular enzymes and proteins. Furthermore, free radicals could initiate the peroxidation of membrane bound PUFAs, leading to both functional and structural myocardial injury [11]. Free radicals generated by isoproterenol causes alterations in membrane integrity, permeability of mitochondria and these alterations results in the changes in mitochondrial membrane structure and function, with inactivation of TCA cycle enzymes and an altered mitochondrial respiration.

Current medical therapies for MI are aimed at suppressing neurohormonal activation (e.g., angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, β-adrenergic receptor antagonists, and aldosterone receptor antagonists) and treating fluid volume overload and hemodynamic symptoms (diuretics, digoxin, inotropic agents). Therefore, there is a need that experience based empirical knowledge if coupled with elucidation of the exact chemical in the plant responsible for therapeutic action could provide a scientific basis to the herbal drugs and increase their acceptability.

Such scientifically generated data will project herbal medicine in a proper perspective and help to sustain the global market [12]. In the Ayurvedic literature "Sarangdhar Samhita" dated centuries ago in 1300 AD. has highlighted the concept of poly-herbalism in this ancient medical system Srivastava. Polyherbal Formulations are known to express high effectiveness in a vast number of diseases. The therapeutic effect of herbal medicines is exerted due to the presence of different phytoconstituents and the effects are further potentiated when compatible herbals are formulated together [13-15]. Due to the fact that Polyherbal Formulations are a product of the nature, they are relatively cheaper, eco-friendly and readily available than allopathic drugs [16].With the above pharmacological facts on hand, nine medicinal plants such as *Punica granatum (rind), Catharanthus roseus, Gymnema sylvestre, Cissus quadrangularis, Garcinia cambogia, Tinospora cordifolia, Terminalia arjuna, Urginea indica, Ficus racemosa* was selected for the present study which has antioxidant, hypocholestremic, free radical scavenging activity, cardiotonic activity and further more.

Here we investigate the cardioprotective effects of PH crude extract on myocardial mitochondrial function in isoprenaline-induced myocardial infarction in rats, with respect to changes in the activity of TCA cycle enzymes and histopathological studies in the heart tissue.

Materials and Methods

Plant collection and identification

The plants and fruits are collected in and around Coimbatore. They are authenticated by Botanical Survey of India (BSI) in "Tamil Nadu Agriculture University" Coimbatore. Various parts of the plant such as fruit rind, flowers, leaves and bark are used for the current study.

Extraction and preparation of polyherbal drug formulation

Each one gm of a poly herbal (PH) formulation contains equal amount of *Punica granatum* (rind), *Catharanthus roseus* (leaves), *Gymnema sylvestre* (leaves), *Cissus quadrangularis* (leaves and stem), *Garcinia cambogia* (fruit), *Tinospora cordifolia* (dimber), *Terminalia arjuna* (bark), *Urginea indica* (bulb), *Ficus racemosa* (fruit and leaves). 10 g of the dried powder of each plant was taken and cold macerated with hydro-ethanolic solvent with occasional stirring for 3 days. After 3 days, the suspensions were filtered through a fine muslin cloth and the filtrate was evaporated to dryness at low temperature (<400°C) under reduced pressure in a rotary evaporator. The yield of crude extract is called as polyherbal (PH) extract which was found to be 9.64% and were stored in an air-tight desiccator's and used for further analysis. Finally, dark brown colored crystals of approximately 25 g were obtained [17,18].

Experimental animals

Male albino rats of Wistar strain weighting about 130-150 g obtained from the animal breeding station, Thrissur, Kerala, India were used for the study. The animals were maintained under standard conditions of humidity, temperature (25 ± 20°C) and light (12 hours light/dark). They were acclimatized to animal house conditions and were fed on a commercial pellet rat chow (AVM cattle Feeds, Coimbatore, Tamilnadu) and water ad libitum. Experimental animals were handled according to the university and Institutional Legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India and the proposal number is KMCRET/PhD/15/2015-2016.

Drugs and chemicals

Propranolol was taken as marketed tablets by Sun Pharmaceuticals Ltd. Isoprenaline hydrochloride was purchased from Sigma Aldrich Co, St, and Louis, USA. All other chemicals are of analytical grade, purchased from Merck, SD Fine chemicals, Qualigens and Hi Media Pharmaceuticals.

Experimental procedures

A total of 48 animals were used and divided into eight groups containing 6 animals each.

Group 1: The rats received only standard rat pellet for 30 days. The animals serve as healthy controls.

Group 2: The rats were administered Isoproterenol (20 mg/100 g Bwt) administered subcutaneously twice at an interval of 24 hours dissolved in normal saline.

Group 3: The rats were pretreated with PH extract (250 mg/kg Bwt) for period of 30 days and isoproterenol (20 mg/100 g Bwt) subcutaneously twice at an interval of 24 hours at the end of treatment period on the 29th and 30th days.

Group 4: The rats were pretreated with PH extract (500 mg/kg Bwt) for period of 30 days and isoproterenol (20 mg/100 g Bwt) subcutaneously twice at an interval of 24 hours at the end of treatment period on the 29th and 30th days.

Group 5: The rats were pretreated with Propranolol (10 mg/Kg Bwt) for a period of 30 days and isoproterenol (20 mg/100 g) subcutaneously twice at an interval of 24 hours at the end of treatment period on the 29th and 30th days.

Group 6: The rats were pretreated with Propranolol (20 mg/kg Bwt) for period of 30 days and isoproterenol (20 mg/100 g) subcutaneously twice at an interval of 24 hours at the end of treatment period on the 29th and 30th days.

Group 7: The rats were treated with PH extract (250 mg/kg Bwt) for periods of 30 days.

Group 8: The rats were treated with PH extract (500 mg/kg Bwt) for periods of 30 days.

After the last treatment, all the rats were sacrificed by cervical decapitation. Heart tissue was excised immediately and rinsed in ice chilled normal saline. One gram of heart tissue was taken and Homogenized with 0.1M cold buffer (pH 7.4) in a potter Homogenizer fitted with a Teflon plunger at 600 revolutions per for 3 min. The homogenate was used for various biochemical assays.

Biochemical assay (TCA cycle enzymes)

The activities of Isocitrate dehydrogenase (ICDH) were estimated by the method [19]; Succinate dehydrogenase (SDH) was estimated by the method [20]; Malate dehydrogenase (MDH) was estimated by the method [21]; α- keto-glutarate DHase was estimated by the method [22]; NADH dehydrogenase was estimated by the method [23].

Histopathological study

Animals were sacrificed on the day of withdrawal of blood; hearts were removed, washed immediately with saline and then fixed at 10% buffered formalin. The hearts stored at 10% buffered formalin were embedded in paraffin, sections cut at 5 mm and stained with hematoxylin and eosin. These sections were then examined under a

light microscope for histoarchitectural changes [24].

Statistical Analysis

Results are expressed as mean ± SD. The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using a statistical package program (SPSS 10.0 for windows), taking P<0.05 as significant [25].

Results

Effect of extracts on the TCA cycle enzymes

The activities of TCA cycle enzymes such as Isocitrate dehydrogenase, Malate dehydrogenase, Succinate dehydrogenase, NADH dehydrogenase and α-Ketoglutarate dehydrogense in the heart of control and experimental rats are shown (Table 1). Mitochondria, the main consumers of molecular oxygen in the cardiac cell that link energy releasing activities of electron transport and proton pumping with the energy conserving process of oxidative phosphorylation, to harness the value of foods in the form of adenosine triphosphate. Electron transport and oxidative phosphorylation alone require the coordinated action of five enzyme complexes, which together are composed of different structural proteins. The isoproterenol induced myocardial rats of Group II (20 mg/100 g Bwt) of Isocitrate dehydrogenase, Malate dehydrogenase, Succinate dehydrogenase, NADH dehydrogenase, α-Ketoglutarate dehydrogense shows a significant (P<0.05) decline when compared to normal control rats of Group I.

Histopathological effect on the tissue of heart

The effect of the PH crude extract on the histological changes in the myocardial tissues in normal and Isoproterenol induced rats is shown in Figures 1-4 (Group: I, IV). The histopathology of heart tissue section of control rats (Group I) showed the normal cell structure with the normal architecture of the myocardium. The rat groups were injected with isoproterenol (Group II) had shown the necrosis of myofibrils and edema through the penetration of inflammatory cells and extravasation of RBC. This characteristic change is due to the disruption of muscular damage of isoproterenol. The ROS are responsible or the release of lysosomal enzymes to the cytosol leading to the further myocardial cellular injury during myocardial Infarction. Isoproterenol induction was found to increase lysosomal hydrolase activities for tissue damage as well as altering the fragility of the lysosomal membrane of cardiomyocytes. A section of inflammatory cells of rats (Group VII and VIII) treated only with PH crude extract (250 and 500 mg/Kg Bwt) showed the normal architecture and no inflammation, no focal myocardial necrosis in a focal area (Group VII and Group VIII). However, the PH crude extract did not alter cardiac cell structure. Thus, the effect of an oral dose of 500 mg/Kg Bwt of PH crude extract was more efficacious than the dose of 250 mg/Kg Bwt. This study reveals that the PH crude extract may be due to the synergistic action of the phytochemicals which are contributed through the free radical scavenging or antioxidants (*in vivo*) in the treated groups. Thus, these observations prove the additional supportive evidence of the cardioprotective effect.

Discussion

The TCA cycle enzymes are located in the outer membrane of mitochondria and could be pretentious by excessive production of free radicals by isoproterenol [26,27]. Inhibition of these enzymes by ROS may affect the mitochondrial substrate oxidation, resulting in reduce oxidation of substrates, reduced rate of transfer of reducing equivalents to molecular oxygen and depletion of cellular energy [28]. Succinate dehydrogenase is an integral membrane protein which is tightly attached to the inner membrane and is directly linked to the electron transport, transferring electrons to the respiratory. It is a site for metabolic control in the TCA cycle and contains many cysteine rich sulfur clusters and can be inhibited by agents that modify sulfhydryl groups. Isoproterenol administration is known to alter protein-bound sulfhydryl groups and hence might have resulted in the inactivation of the enzyme [29]. NADH dehydrogenase is present in the inner mitochondrial membrane and are involved in the synthesis of high energy compound ATP, which needs a requirement of cardiolipin. This could be due to the free radical induced destabilization of mitochondrial membrane or due to the enhanced phospholipid degradation and non-availability of cardiolipin for its functional activity [30,31].

An increase in proteolytic activity during isoproterenol induction may also be responsible for the decreased MDH activity [32]. The increased production of free radicals in mitochondrial cells in the tissue, also a decrease in oxygen consumption, respiratory ratio was observed in mitochondria is also one of the reasons for the decrease in MDH activity in Group II [33]. The Isocitrate dehydrogenase (ICDH) catalyzes oxidative decarboxylation of isocitrate to α-ketoglutarate and require either NAD+ or NADP+ producing NADH and NADPH, respectively [34]. Therefore, ICDH may play an antioxidant role during oxidative stress. ICDH is involved in the supply of NADPH needed for GSH production against mitochondrial and cytosolic oxidative damage [35,36]. Hence, the damage of ICDH may result in the perturbation of the balance between oxidants and antioxidants and subsequently lead to a pro-oxidant condition. The activity of ICDH can be inhibited by glycation of ICDH. Reactive oxygen species contribute to the inactivation of ICDH by glycation [37].

Biological membranes and subcellular organelles are rich in PUFA

Treatment Groups	ICDHase	MDHase	α-ketolutarate DHase	SDHase	NADH- DHase
Group I	647.19 ± 4.56^{a}	318.62 ± 0.23^{a}	107.37 ± 0.69^{a}	235.90 ± 1.45^{a}	160.05 ± 4.08^{a}
Group II	438.86 ± 14.17^{b}	190.60 ± 0.24^{b}	69.40 ± 0.30^{b}	125.02 ± 1.23^{b}	83.87 ± 1.35^{b}
Group III	480.84 ± 12.19^{c}	285.40 ± 7.50^{c}	70.80 ± 6.16^{c}	105.10 ± 3.76^{d}	124.93 ± 2.12^{c}
Group IV	666.63 ± 11.73^{d}	297.25 ± 5.37^{d}	85.58 ± 2.95^{d}	177.62 ± 1.61^{c}	147.45 ± 2.18^{d}
Group V	480.50 ± 11.27^{c}	285.90 ± 3.77^{c}	70.27 ± 4.28^{c}	105.82 ± 3.11^{d}	124.78 ± 2.15^{c}
Group VI	666.92 ± 11.27^{d}	297.03 ± 0.81^{d}	85.17 ± 3.90^{d}	177.92 ± 2.86^{c}	147.43 ± 5.15^{d}
Group VII	647.89 ± 13.92^{a}	318.75 ± 0.55^{a}	107.14 ± 2.74^{a}	235.67 ± 3.52^{a}	160.20 ± 4.03^{a}
Group VIII	647.85 ± 12.02^{a}	318.72 ± 0.70^{a}	107.08 ± 3.49^{a}	235.40 ± 3.23^{a}	160.12 ± 3.00^{a}

$^{a-f}$ Mean values with in a column of common superscript means Non-significant; Values are mean ± SD of six samples in each group; ICDH (Isocitrate dehydrogenase)- nmol of α-keto-glutarate formed/min/mg of protein; MDH (Malate dehydrogenase)- μ mol of NADH oxidized/min/mg of protein; α-k-G DHase (alpha-ketoglutarate dehydrogenase)- nmol of ferrocyanide formed/min/mg of protein; SDH (Succinate dehysdrogenase)- μ nmol of succinate oxidised/min/mg of protein; NADH-DHase- μ mol of NADH oxidised/min/mg of protein; $^{a-f}$Mean values with in a column no common superscript differs significantly at (<0.05) by DMRT

Table 1: Effect of hydroethanolic poly herbal formulation (PHF) extract on TCA cycle enzymes.

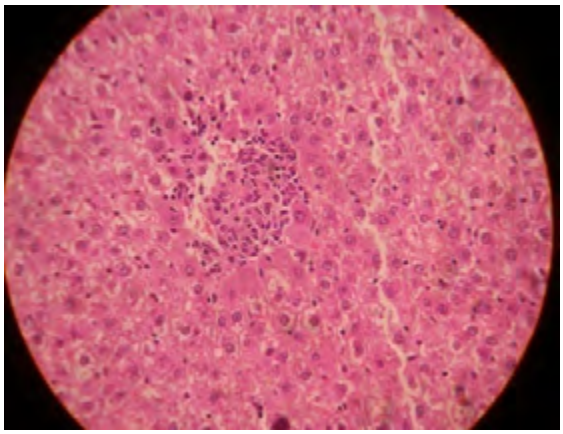

Figure 1: Normal control heart showing normal myocardial myocardial architecture (Group I).

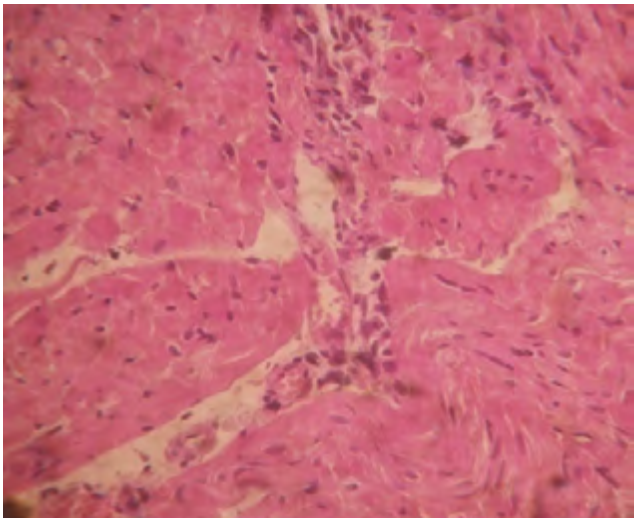

Figure 2: Isoproterenol induced (20 mg/100 g Bwt) rats shows necrosis (NC) of myofibrils and edema through Penetration of inflammatory cells (IC), normal and extravasation of RBC (Group II).

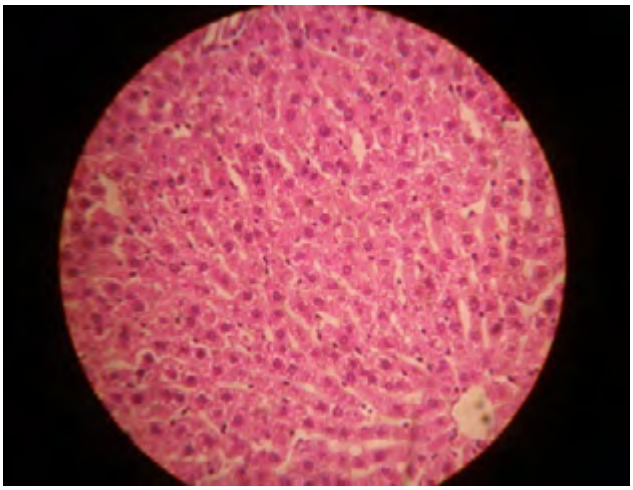

Figure 3: ISO (10 mg/100 g Bwt)+PH crude extract Pretreatment (250 mg/Kg Bwt) showing lesser necrosis (LMNC) and edema (Group III).

are the major sites for free radical mediated damage. Activation of LPO in mitochondria corresponds with changes in lipid composition which includes a decrease in the level of total and readily oxidizable lipid, Cardiolipin [38]. These results are in accordance [39], who has reported the diminished activities of TCA cycle enzymes in Doxorubicin induced rats along with Nicorandil. Pretreatment with the hydroethanolic PH crude extract of Group III and Group IV (250 mg/kg, 500 mg/kg Bwt) for a period of 30 days showed a significant ($p<0.05$) elevation in the Isocitrate dehydrogenase, Malate dehydrogenase, Succinate dehydrogenase, NADH dehydrogenase and α-Ketoglutarate dehydrogense. This protection against isoproterenol which inevitably suggest that the phytochemicals are very efficient in maintaining the mitochondrial membrane integrity. Our previous studies have showed that the PH crude extract is well known to counteract reactive oxygen species and to increase antioxidant defense status in experimental animals [18]. The medicinal plants have also been reported to increase the GSH content and to protect the reduced glutathione from the free radical attack which helps to maintain the activity of enzymes like dehydrogenases that requires or possesses sulfhydryl groups at the active site. The membrane stabilizing property of medicinal plant is already recognized. Since TCA cycle enzymes are located in the mitochondrial inner membrane and matrix region, the ability of the PH crude extract to maintain the mitochondrial membrane intact offers an important protective mechanism against isoproterenol-induced alterations in the mitochondrial architecture. Also, may be due to the PH crude extract contain the enormous level of phytoconstituents could synergistically act on the energy producing affected pathway. Flavonoids especially quercitin and phytic acid which has the free radical scavenging property [18]. Phytic acid contains the calcium, magnesium and potassium which inhibit the phospholipid degradation in the biological membranes, thereby maintains the level of Cardiolipin in the membrane, thereby increases the ATP production which increases the TCA cycle enzymes [27].

This observation of a hydroethanolic PH crude extract treatment is supported on the reduced activities of TCA cycle enzymes were replenished by *Centella asiatica* against isoproterenol induced MI in rats [40]. There is no significant ($p<0.05$) changes in Group V and VI (propranolol 10 mg/kg, 20 mg/Kg Bwt) when compared to Group III and IV (PH crude extract 250 mg/kg and 500 mg/ kg Bwt). Propranolol has not only β-adrenoreceptor blocking action [41,42] but also has other pharmacological effects such as Na^+ channel blocking action Doggrell and an antioxidant effect [43]. This agent is known to protect the myocardium against ischaemia/reperfusion or hypoxia/reoxygenation-induced inury in *in vivo* and *in vitro* experimental animals [44-46]. As the PH crude extract and standard drug propranolol, both are in the equal response in TCA cycle enzymes. This may because, the PH crude extract using nine individual medicinal plants, in which *Terminalia arjuna, Tinospora cardifolia* and *Urginea indica* were already reported for its antioxidant activity and mainly cardioprotective activity by preventing the membrane damage and leakage of enzymes [47-50]. The result showed the protective role of hydroethanolic PH crude extract act as a free radical and ROS scavengers in the electron transport chain of mitochondria. This indicates that deranged energy metabolism in Isoproterenol induced MI was rectified and favorable restoration of mitochondrial TCA cycle enzymes. The hydroethanolic PH crude extract has shown its capability of protecting the myocardium against Isoproterenol induces rats.

In this context, observation made in the present study was supported, who have noticed the similar histological changes of heart tissues in Isoproterenol induced Myocardial infarction in rats. Similar

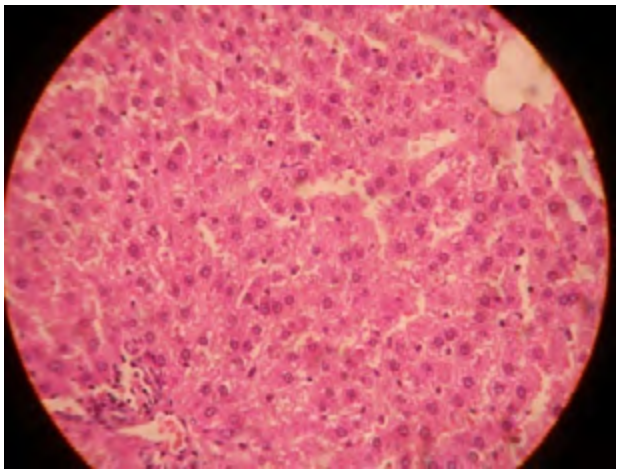

Figure 4: ISO (10 mg/100 g Bwt)+PH crude extract pretreatment (500 mg/kg Bwt) showing lesser myocardial myocardial necrosis and edema with conical myocardium (CM) and showed healthy myofibres (Group IV).

results have been shown by several studies [51,52]. A section on heart tissues of rats (Group III and IV) treated with PH crude extract (250 mg/Kg and 500 mg/Kg BWt) showed a very mild inflammation and focal myocardial necrosis (at one place) when compared with Group II. Reverse these changes of muscle cells and focal cardiac fibers and a well prevented normal morphology of cardiac muscle with minimal lesions. This may be due to the phytoconstituents such as flavonoids, tannins, alkaloids and mainly glycosides and antioxidant profile. In connection with this study, who have observed the similar histological effects of *Bombyx mori L Cocoon* in isoproterenol induced myocardial infarction [53], who have also noticed the similar recovery in the treatment with *Rhododendron arboreum* in Isoproterenol induce myocardial rats.

The section of heart tissues of isoproterenol induced rats (Group V and Group VI) treated with standard drug propranolol (10 and 20 mg/Kg Bwt) showed the myocardial tissues with lesser myocardial necrosis and edema along with mild inflammation than Group III and IV (250 mg/Kg Bwt and 500 mg/Kg Bwt) of PH crude extract treated. This histological changes were due to the inhibitory effect of propranolol, arresting the β- blockers. In this connection, propranolol is a β- blocker which blocks the β-blocking agent [54]. Propranolol is a liphobilic β-blocker in nature, β- adrenergic blockers have long been useful adjuvants in the management of myocardial necrosis [55]. The research studied on *Moringa oleiferalam* in isoproterenol induced Myocardial Infarction and similar to these results various authors have reported the effect of propranolol, such as in Isoproterenol induced MI in rats [56]. The higher 20 mg/Kg Bwt of the propranolol treated animals shows a mild inflammation and necrosis when compared with 10 mg/Kg Bwt of propranolol treated animals.

Conclusion

In conclusion, the present study demonstrated that the Poly herbal formulation (PHF) crude extract exhibit synergistic cardioprotective activity revealed by improving the TCA cycle enzyme and protective effect on myocardium like the reference drug propranolol. Hence, It might help in preservation of mitochondrial energy metabolism as well as the generation of myocardium. PH crude extract has a significant therapeutic beneficial effect on the protection of heart against isoproterenol induced Myocardial Infarction, possibly through inhibiting LPO and cytokine levels or cardiolipin. The mechanism might underlying cardioprotective effect of the PHF crude extract may be attributed to the preservation of mitochondrial function during myocardial infraction, probably via activation of mitochondrial energy metabolism. The study of this PH crude extract on coronary heart disease play a vital role in managing cardiac diseases and also attain a therapeutic value.

Acknowledgements

The authors are grateful to the Principal, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India.

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