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EFFECT OF SEEDS OF *SPERMACOCE HISPIDA LINN* ON MITOCHONDRIAL ENERGY PRODUCTION IN ISOPROTERENOL INDUCED MYOCARDIAL INFARCTED RATS

R. Dhevi[1]*, V. Elango[2]

[1]*Research Scholar, Department of Siddha Medicine, Tamil University, Thanjavur, TN, India.*
[2]*Assistant Professor, Department of Siddha Medicine, Tamil University, Thanjavur, TN, India.*

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ABSTRACT

Mitochondria play a central role in molecular events leading to tissue damage in ischemia. The present study aimed to investigate the effects of pretreatment with S*permacoce hispida* seed extract on isoprenaline-induced myocardial infarction in heart mitochondrial function in experimental rats. Two different doses of the seed extract such as 100 and 200 mg/kg body weight was used to prove the cardioprotective effect against 100mg/kg body weight of isoproterenol (ISO) which was administered subcutaneously twice at an interval of 24 hours. ISO induced cardiotoxicity caused a significant decrease in the activities of TCA cycle enzymes such as Isocitrate dehydrogenase (ICDH), Malate Dehydrogenase(MDH), succinate dehydrogenase (SDH), NADH dehydrogenase and Cytochrome-C-oxidase in heart mitochondria in rat model. Pretreatment with *Spermacoce hispida* seed extract attenuated these mitochondrial alterations and restored the TCA cycle enzyme activities to near normal values. The present findings indicate that the protective effect of seeds of *Spermacoce hispida* can be attributed to the activation of mitochondrial energy metabolism. The plant may have important implication for future therapeutic approaches involving in the prevention of coronary heart disease. This study identifies the membrane protective action of HAE pre treatment.

Corresponding author
R. Dhevi
Research Scholar,
Department of Siddha medicine,
Tamil University,
Thanjavur-613 010
TamilNadu, India.
rdhevi23@gmail.com
(0)9952253899

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INTRODUCTION

Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy [1]. 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 [2]. Mitochondria are generally considered as origin but also as target for reactive oxygen species [3,4]. 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 [5].

In this study free radicals generated from isoproterenol (ISO) causes alterations in membrane integrity and permeability of mitochondria. In addition, the positive inotropic and positive chronotropic response of ISO augments myocardial oxygen consumption [6]. These alterations result in altered electrolyte levels including calcium entry, which results in phospholipase activation, further leading to ATP depletion and irreversible injury [7]. There is close association between ATP depletion and the metabolic changes on the onset of swelling, loss of ionic gradients and, alterations in mitochondrial membrane structure and function, with inactivation of TCA cycle enzymes and an altered mitochondrial respiration [8].

The purpose of the study is to identify the cardioprotective effect of *Spermacoce hispida* in Isoproterenol induced myocardial infracted rat model. *Spermacoce hispdia* Linn commonly known as 'Shaggi button weed' belongs to the family *Rubiaceae* and is widely distributed throughout the world as an useful medicinal plant [9]. The seed of the plants are rich in flavonoids in both *invitro* and *invivo*. All the parts of the plant have an ethno medical importance [10]. It has been also reported that the plant is an effective natural drug for the treatment of hypertension and it has hepatoprotective, anti inflammatory and antioxidant properties. The present study highlights the efficacy of seed extract of *Spermacoce hispida* on the alterations of mitochondrial energy metabolism in ISO induced myocardial damage.

MATERIALS AND METHODS

Animals:

Adult male albino wistar rats weighing 150-250g were obtained from Sri Venkateshwara Enterprises, Bangalore-560021, India. The animals were housed in polypropylene cages. They were fed with standard diet and water *ad libitum* and housed under standard environmental conditions.

Experimental design:

Group1: The rats of group 1 serve as control and they did not receive any treatment
Group 2: Rats were administered with ISP (100mg/kg b.wt) dissolved in 0.9% saline subcutaneously twice at the interval of 24 hours [10].
Group 3: Rats were administered with 100mg/kg body wt. of *Spermacoce hispida* seed extract for 45 days. ISP was injected subcutaneously on 45th day.
Group 4: Rats were administered with 200mg/kg body wt. of *Spermacoce hispida* seed extract for 45 days. ISP was injected subcutaneously on 45th day
Group 5: Rats were administered with 100mg/kg body wt. of *Spermacoce hispida* seed extract alone for 45 days.
Group 6: Rats were administered with 200mg/kg body wt. of *Spermacoce hispida* seed extract alone for 45 days.
Group 7: Rats were administered with Vitamin E at 100 mg/ kg b.wt. for 45 days. ISP was injected subcutaneously on 45th day.

Induction of Myocardial infarction:

MI was induced in rats by subcutaneous injection of 100 mg/kg isoprenaline hydrochloride dissolved in saline once daily for two successive days [12, 13].

Isolation of Mitochondria

Heart mitochondrion was isolated initially [14]. The heart tissue was homogenized in ice cold 50 mM Tris–HCl (pH 7.4) containing 0.25 M sucrose. The homogenates were centrifuged at 700Xg for 20 min, and then, the supernatants obtained were centrifuged at 9000Xg for 15 min. The obtained pellets were washed with 10 mM Tris–HCl (pH 7.8) containing 0.25 M sucrose and finally resuspended in the same buffer.

Biochemical analysis

Mitochondrial pellet was suspended in 0.25 M sucrose containing 10 mM Tris–HCl (pH 7.4) and 1 mM EDTA to a known volume (2 ml) and used for the following enzyme estimations. The activities of Isocitrate dehydrogenase (ICDH) was estimated by the method of King, 1965b [15], Succinate dehydrogenase (SDH) was estimated by the method of Slater and Bonner, 1952 [16], Malate dehydrogenase (MDH) was estimated by the method of Mehler, 1948 [17], and cytochrome-C-oxidase was estimated by the method of Pearl [19]. The mitochondrial protein content was also estimated by Lowry's Folin phenol method [20].

NADH dehydrogenase was estimated by the method of Minakami, 1962 [18]. The reaction mixture contained 1.0 ml of phosphate buffer, 0.1 ml of potassium ferricyanide, 0.1 ml of NADH and 0.2 ml of mitochondrial suspension. The total volume was made up to 3.0 ml with water. NADH was added just before the addition of the enzyme. A control was also treated similarly without NADH. The change in OD was measured at 420 nm as function of time for 3 min at an interval of 15 seconds. The activity of NADH dehydrogenase was expressed as µM of NADH oxidized/hr /mg protein.

Statistical analysis:

The data were analysed by using One way ANOVA followed by DMRT. The results from experimental groups were compared with respective control and *p* values < 0.05 were considered statistically significant.

TABLE: Effect of HAE on Mitochondrial enzymes in ISO administered rats.

GROUPS	ICDH	MDH	SDH	NADH DH	Cyto-c oxidase
Normal	3.14 ± 0.69 ^{b}	225.20 ± 25.9 ^{e}	120.7 ± 18.9 ^{b}	139.45±15.5b	0.32 ± 0.021 b
ISO	1.79 ± 0.40 ^{a}	48.2 ± 6.4 ^{a}	58.2 ± 18.7 ^{a}	89.12 ± 2.51a	0.23 ± 0.012a
100 mg/kg b.wt. HAE + ISO	2.20 ± 0.47 ^{ab}	160.1 ± 45.8 ^{c}	60.7 ±25.9 ^{a}	122.69 ± 19.35c	0.29± 0.01a
200 mg/kg b.wt. HAE + ISO	3.17 ± 0.89 ^{b}	175.3 ± 43.4 ^{d}	113.8 ± 31.2 ^{b}	136.41 ± 13.58b	0.30 ± 0.02b
100 mg/kg b.wt. HAE	3.11 ± 1.2 ^{b}	195.2 ± 25.8 ^{e}	110.5 ± 18.1 ^{b}	135.19±20.9c	0.31± 0.024b
200 mg/kg b.wt. HAE	3.02 ± 1.1 ^{b}	201.2 ± 30.4 ^{e}	114.1 ± 18.9 ^{b}	140.23±15.7b	0.32± 0.01b
Vitamin E + ISO	3.09 ± 0.49 ^{b}	104.1 ± 16.3 ^{b}	89.2 ± 19.8 ^{ab}	132.58±24.9c	0.32 ± 0.021b

Note: Activity of ICDH, MDH, SDH, NADH DH and Cyto C-Oxidase are represented as nM of α-ketoglutarate formed/hr/mg of protein, nM of NADH oxidized/hr/mg of protein, nM of succinate oxidized/hr/mg of protein, nm of NADH oxidized/min/mg of protein and nmol/min/mg protein. Values are Mean ± SD (n=6). Significant difference was observed between different groups using One Way ANOVA followed by DMRT. Values with different letters like a,b,ab,c,d,e of same column are differ significantly (P<0.05).

RESULT

The activities of the TCAcycle enzymes in the control and experimental groups are displayed in Table. The activity of the malate dehydrogenase ($p < 0.05$), isocitrate dehydrogenase ($p < 0.01$), succinate dehydrogenase ($p < 0.01$), NADH dehydrogenase ($p < 0.01$) and cytochrome-Coxidase ($p < 0.05$) were found to be significantly lower in the mitochondria of Group II rats subjected to ISO exposure as compared with those of control (Group I). Rats pretreated with Spermacode hispida seed extract (Group III&IV) significantly attenuated the alterations induced by ISO when compared with Group II animals. Prior oral treatment with SH alone (Group V&VI) registered no significant changes when compared to control. Rats treated with Vitamin E and ISO (Group VII) also shows similar activity of SH treated rats.

DISCUSSION

Mitochondria, the main consumers of molecular oxygen in the cardiac cell, 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. Free radicals generated from ISO causes alterations in membrane integrity and permeability of mitochondria. In addition, the positive inotropic and positive chronotropic response of ISO augments myocardial oxygen consumption [6]. These alterations result in altered electrolyte levels including calcium entry, which results in phospholipase activation, further leading to ATP depletion and irreversible injury [7]. There is close association between ATP depletion and the metabolic changes on the onset of swelling, loss of ionic gradients and, alterations in mitochondrial membrane structure and function, with inactivation of TCA cycle enzymes and an altered mitochondrial respiration [8].

In the present study, the activity of TCA cycle enzymes like Isocitrate dehydrogenase (ICDH), Malate dehydrogenase (MDH), Succinate dehydrogenase (SDH), Cytochrome-c-oxidase and NADH Dehydrogenase is found to be decreased significantly in mitochondrial fraction of diseased animals against normal animals (Table). Treating animals with HAE of seeds of *Spermacoce hispida* is observed to increase the activity of the TCA cycle enzymes against diseased rats significantly. The activity exponentially increased dose dependently against normal animals. Significant difference has not been observed in Group V and VI animals which are treated with only HAE at various doses against Group I rats. This confirmed the ISO induced mitochondrial damage and membrane protection exhibited by HAE pretreatment.

Electron transport and oxidative phosphorylation alone require the coordinated action of five enzyme complexes, which together are composed of different structural proteins. A reduction in enzyme content could arise in a failure of assembly of electron transfer chain complexes or enhanced rates of degradation of complexes.

ISO treatment may be attributed to induce loss of mitochondrial calcium which is required for the stimulation of dehydrogenases and thereby, decrease the activity of those enzymes [21]. The increased LPO has been reported to alter the lipid environment of the membrane from the damaged mitochondria and the TCA cycle enzymes may be released in to the cytoplasm [22]. This may be the reason for decreased activity of TCA cycle enzymes in the mitochondria. The reason for decreased activity of mitochondrial enzymes in ISO administered rats as enhanced phospholipids degradation resulting in the non-availability of cardiolipin required for their functional activity [23].

CONCLUSION

In conclusion, the present result has shown that the seeds of *Spermacoce hispida* are capable of protecting the myocardium against ISO induced injury and enhancing the recovery of myocardial high-energy phosphate content. The mechanism underlying cardioprotective effect of *Spermacoce hispida* may be attributed to the preservation of mitochondrial function during heart failure, probably via activation of mitochondrial energy metabolism. The study of this seed extract on coronary heart disease play a vital role in managing cardiac diseases and also attain a therapeutic value.

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