ISSN (Online) 2249-6084 (Print) 2250-1029

International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)
[Impact Factor – 0.852]

Journal Homepage: www.eijppr.com

Research Article

Cardiotoxic Activity of Leaves Extract of *Raphanus sativus* Linn. in Adult Male Albino Rats

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Article info

Article History:
Received 27 March 2014
Accepted 22 April 2014

Keywords:
Raphanus sativus,
Cardiotoxic effect,
Isoproterenol

Abstract

The present study was designed to investigate whether *Raphanus sativus* might protect the heart against myocardial injury induced by isoproterenol (ISO) on the basis of its effect on biochemical and histological parameters. Adult male albino rats (180-200 g) were divided into four groups: normal control, isoproterenol control (ISO), RSLE control and RSLE+ISO. *R. sativus* was administered at 500 mg/kg, *p.o.* for 29 days. On 28th and 29th day, the animals of ISO control and RSLE+ISO were administered isoproterenol (85 mg/kg, *s.c.*). On the 30th day, rats were sacrificed and the levels of cardiac marker enzymes (aspertate aminotransferase; AST, alanine aminotransferase;ALT, lactate dehydrogenase; LDH and creatine phosphokinase; CPK) were assessed in serum. Heart specimens were processed for histopathological study. Administration of *R. sativus* significantly ($p<0.01$) increased the serum cardiac marker enzymes in RSLE and RSLE+ISO group. Histopathological study also provides evidence for the cardiotoxicity of *R. sativus* leaves extract in RSLE and RSLE+ISO treated rats.The present results suggests that *R. sativus* increased the cardiotoxicity alone and in ISO treated rats. This could be due to the histaminergic activity leading to increased myocardial biomarkers. Further studies are needed in understanding and to confirm the exact mechanism of cardiotoxic effect of *R. sativus*.

1. INTRODUCTION

Cardiovascular diseases (CVD) are leading causes of deaths despite several advancements in the medical interventions. Among these, the ischemic heart diseases, acute myocardial infarction (MI) in particular are the most alarming[1]. CVDs in India cause 3 million deaths per year, accounting for 25% of all mortality. The WHO predicts that deaths due to circulatory system diseases are projected to double between 1985 and 2015[2]. MI contributes to approximately 16% of all deaths in developing countries and 50% in developed countries[3]. Acute myocardial necrosis is accompanied by increased cardiac marker enzymes, accumulated lipid peroxides, ischemic electrocardiograph, reduced cardiac function and the patient may experience significant disability or die[4, 5].

Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist, and has been found to induce myocardial injury in rat resulting in infarct like necrosis of the heart muscles[6]. Some of the mechanisms proposed to explain the Isoproterenol-induced injury to myocardial cells include hypoxia[7], calcium overload[8], depletion of energy reserves[9] and excessive productionof free radicals resulting from oxidative metabolism of catecholamines[10]. The pathophysiological and morphological alterations in the heart of this non-coronarymyocardial necrotic rat model are similar to those taking place in human myocardial infarction[11, 12].

Epidemiological, clinical and experimental studies have provided compelling evidence that MI is largely preventable by antioxidant intervention via suppression of free radical generation and/or augment endogenous antioxidant[13]. There has been a tremendous effort to develop non-toxic and safe antioxidants from natural products to minimize the damage to the heart. Medicinal plants and their constituents have gained great attention for their salutary effects and potential to treat many CVDs[14].

Raphanus sativus have several pharmacological effects such as Gut stimulatory[15], Antifungal[16], Spasmogenic[17], Antilithiasic and Hypolipidaemic[18], Anti-Inflammatory[19], Anticarcinogenic[20], Hepatoprotective[21], Antiurolithiatic[22], and Gastroprotective[23] activity. HPLC identification of polyphenolics indicated the presence of catechin, protocatechuic acid, syringic acid, vanillic acid, ferulic acid, sinapic acid, o-coumaric acid, myricetin, and quercetin in leaves and stem[24].

Isoproterenol induced cardiotoxicity results in a marked elevation of serum marker enzymes (aspertate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK)[25] and decrease in the biomarkers indicate the cardio protective activity of the leaves extract. In the study, we examined the cardio protective effect of *R. sativus* against isoproterenol induced toxicity in rats.

2. MATERIALS AND METHODS

2.1 Extraction of the Crude Drug

The shade dried leaves were extracted with 70% ethanol after defatting with petroleum ether by Soxhelet extraction. The extract was stored between 2 and 8ºC for further studies. The extract was subjected to qualitative phytochemical tests using standard procedure.

2.2 Drugs

Isoproterenol (ISO): Isoproterenol hydrochloride was purchased in a white powder form from Sigma Aldrich, USA. It was dissolved in distilled water and used immediately for subcutaneous administration.

2.3 Experimental animals

Twenty four adult male albino rats (180-200 g) were obtained from the animal house of Mallige College of Pharmacy. The study protocol was reviewed and approved by theInstitutional Animal Ethics Committee in accordance with CPCSEA guidelines. The

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animals were acclimatized in departmental animal house and housed under standard laboratory conditions of temperature at 25 ± 2ºC, relative humidity of 55 ± 10% and light: dark cycle of 12 hr photoperiod and fed with standard diet and water *ad libitum*. All experiments were performed between 9.00 and 16.00 hr.

2.4 Induction of myocardial injury

Myocardial injury was induced in experimental rats by injecting 85 mg/kg of ISO subcutaneously daily for 2 consecutive days.

2.5 Experimental Design

The experimental animals were divided into four groups of six rats each.

Group I (Control group): rat received vehicle (5 ml/kg, *p.o.*) for 29 days followed by normal saline (1 ml/kg, *s.c.*) on 28th and 29th day.

Group II (ISO control): rat received vehicle (5 ml/kg, *p.o.*) for 29 days daily followed by isoproterenol (85 mg/kg, *s.c.*) on 28th and 29th day.

Group III (RSLE control): rat received *R. sativus* leaves extract (500 mg/kg, *p.o.*) for 29 days followed by normal saline (1 ml/kg, *s.c.*) on 28th and 29th day.

Group IV (RSLE-ISO): rat received *R. sativus* leaves extract (500 mg/kg, *p.o.*) for 29 days followed by isoproterenol (85 mg/kg, *s.c.*) on 28th and 29th day.

R. sativus leaves extract was dissolved in distilled water. Control and ISO treated group received equal quantity of vehicle.

At the end of the experimental period (after 24 hr of second isoproterenol injection). 2 ml blood was collected from the retro-orbital plexus under mild anesthesia. The serum was separated and used for the determination of diagnostic marker enzymes, such as aspertate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) using standard kit procedure. After blood withdrawal, all the rats were sacrificed by cervical dislocation; the heart was dissected out, washed in ice cold saline, weighed after blotting with filter paper and heart weight index (HWI) was computed. Then myocardial tissue was immediately fixed in 10% buffered neutral formalin solution and processed for histopathological studies.

2.6 Statistical analysis

Results are expressed as mean ± SEM. One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Bunferroni test using Graph Pad Prism 5.0 statistical package program (Graph Pad Software, Inc., La Jolla, CA, USA).P-values less than 0.05 were considered as statistically significant.

3. RESULTS

3.1 Phytochemical test

The qualitative phytochemical test revealed the presence of Alkaloids, Glycosides, Saponins, Phytosterol, Flavonoids, Phenols, Protein and free amino acids.

3.2 Effect of *R. sativus* leaves extract on heart weight index (HWI)

The heart weight index of ISO treated rats were significantly ($p<0.01$) increased compared to healthy control group. Similarly, HWI of ISO and RSLE treated rats were significantly ($p<0.001$) increased compared to healthy control group from 2.97 mg/g to 3.67 mg/kg. Whereas, RSLE alone treated in rats shows increased in HWI compared to healthy control group form 2.97 mg/g to 3.06 mg/g. The value of HWI was further increased in RSLE treated animal in ISO induced toxicity.

Table 1: Effect of *R. sativus* leaves extract on heart weight index (HWI) of control and isoproterenol (ISO)-induced oxidative stress and cardiotoxicity in rats.

Group	HWI (mg/g)
I. Healthy control	2.97±0.0244
II. Isoproterenol	3.47±0.0521*
III. RSLE	3.06±0.108
IV.RSLE+ Isoproterenol	3.67±0.137**

*N=6, values are expressed as mean ± SEM, *P<0.01 and **P<0.001 in comparison with healthy control.*

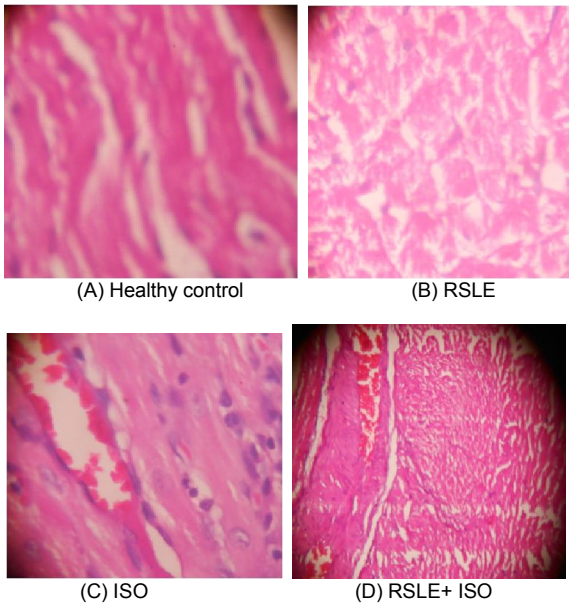
3.3 Effect of *R. sativus* leaf extract on heart architectures

Histopathological study revealed the necrosis of myocytes, and infilteration of inflammatory cells are moderate in rats which received ISO alone, where as it was severe in group treated with ISO and RSLE. Intensity of hemorrhage and inflammation is more in the group treated with RSLE and ISO than as injected with ISO alone.

Table 2: Effect of *R. sativus* leaf extract on heart architectures of control and isoproterenolISO)-induced oxidative stress and cardiotoxicity in rats.

Observation	Control	RSLE	ISO	RSLE+ ISO
Necrosis of myocytes	-	+ +	+ + +	+ + +
Hemorrhage	-	+	+ +	+ + +
Infiltration of inflammatory cells	-	+ +	+ + +	+ + +
Fibroblastic proliferation	-	-	+	+
Hyaline necrosis	-	-	+ +	+ +
Inflammation	-	+ +	+ +	+ + +

Note: Nil (-), Present (+), Moderate (+ +), Severe (+ + +)



(A) Healthy control (B) RSLE

(C) ISO (D) RSLE+ ISO

Figure 1: Structure of myocardial tissues of various treatment groups

3.4 Effect of *R. sativus* leaves extract on cardiac markers enzymes

The biochemical biomarker like AST, ALT, LDH and CPK in ISO treated ratswere found to be increased significantly in comparison to normal control. The AST level has been increased from 58.7 to 128, ALT (46.3 to 153), CPK (375 to 2006) and LDH (519 to 1470 IU/L). The biomarker level of rats treated with *R. sativus* were also increased significantly ($p<0.01$). The AST and CPK level of rats treated with *R.sativus* and ISO were also significantly ($p<0.001$) increased in comparison to ISO only treated group.

Table 3: Effect of *R. sativus* leaf extract on cardiac markers enzymes in the serum of control and isoproterenol (ISO)-induced oxidative stress and cardiotoxicity in rats.

Group	AST (IU/L)	ALT (IU/L)	CPK (IU/L)	LDH (IU/L)
I. Healthy control	58.7±0.67	46.3±1.28	375±1.99	519±1.45
II. ISO	128±8.33	153.0±1.61	2006±99.3	1470±128
III. RSLE	75.5±2.46*	57.0±2.13*	1711±81.5**	1241±37.5**
IV. RSLE+ ISO	175±6.99$^{\#}$	161.0±4.26	2477±89.9$^{\#}$	1615±74.2

*N=6, values are expressed as mean ± SEM, *P<0.01, **P<0.001 in comparison with healthy control, $^{\#}$p<0.01 in comparison with ISO.*

4. DISCUSSION

Isoproterenol (ISO) was used to produce cardiotoxicity in albino rats. ISO is a dual β1-β2 adrenergic receptor agonist that has acute positive chronotropic and ionotropic effects in the heart[26, 27].When administered chronically or at high dosage, ISO has deleterious effect in the heart, inducing hypertrophy[28], necrosis, fibrosis, apoptosis, oxidative damage and inflammatory cell infiltration[29-32]. Histological analysis has shown that ISO induces an infarct-like lesion at the apex of the myocardium[33, 34].

The extract showed the presence of saponin and alkaloids and it was used for Gastroprotective[23] and Hepatoprotective[21] actions. We thought that the extract would show a cardioprotective activity in Isoproterenol induced toxicity. To our surprise, the extract found to induce cardiotoxicity. The cardiac biomarkers like AST, ALT, CPK and LDH have increased indicating the induction of cardiac toxicity by ISO. The RSLE failed to protect the toxicity in spite of having alkaloids and saponins which many times exhibited cardioprotective effects. Surprisingly, the biomarker level was increased after administration of RSLE indicating the cardiotoxic effect.

The biomarkers level increased after the administration of RSLE and ISO probably due to the histamine like action of RSLE on heart. Ghayar and Gilani in their finding reported the histaminergic activation of *R. sativus* mediated through cholinergic receptors[35]. Ghayar in another study on use of *R.sativus* in constipation, they confirm the presence of histaminergic components in *R. sativus* extract[15]. The cardiotoxic activity of *R. sativus* must be due to histaminic activity on heart.

Genovese and Spadaro in their investigation on cardiovascular effects of histamine and H_1-receptor antagonists explained that histamine is stored in large amounts in human cardiac tissue, where it is contained in the cytoplasmatic granules of mast cells. Mast cells are present in normal human heart tissue; they are more abundant in diseased human heart tissue where they lie in close proximity to blood vessels and between myocytes. The histamine content of human heart mast cells is comparable to the histamine content of lung parenchymal and skin mast cells. Consequently, these cells are easily accessible to circulating antigens, drugs and stimuli that activate the cells to release vasoactive mediators which in turn can exert significant cardiovascular effects[36]. Bristow and co-workers in their study confirmed that the release of cardiac histamine is involved in pathogenesis of cardiotoxicity[37].

The extract of *R. sativus* might have stimulated the release of histamine leading to toxicity. Thus the cardiotoxic effects observed in our experiments are may be due to the presence of chemical constituents which triggers the histamine toxicity at cardiac cell.

3. CONCLUSION

The present results suggests that *R. sativus* increased the cardiotoxicity alone and in ISO treated rats. This could be due to the histaminergic activity leading to increased myocardial biomarkers. Further studies are needed in understanding and to confirm the exact mechanism of cardiotoxic effect of *R. sativus*.

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