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Study of Cardioprotective Activity of *Raphanus sativus* L. in the Rabbits

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Abstract: Cardio-pharmacological investigation of *Raphanas sativus* fruit powder, its water and ethanolic extracts was performed in rabbits to search the effective tool against cardiac disorders from natural sources. The fruit powder (0.5-1.5 g kg^{-1} of body weight) tested in Cyclosporin (30 mg kg^{-1}) treated rabbits. The effects of water and ethanolic extracts of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1} of body weight) were also noted in the presence of Cyclosporin. The powder and its aqueous extract highly significantly ($P<0.001$) decreased the Uric acid and activity of enzymes I. e. GOT and LDH in serum of treated rabbits. Ethanolic extract did not show any significant attenuation to the cardiac effects of Cyclosporin in the rabbits. Both the fruit powder and its aqueous extract showed (*in vitro*) free radical-scavenging effect in a dose dependent manner on DPPH assay. *R. sativus* fruit powder and its aqueous extract might strengthen the antioxidant defense system to resist the free radical induced damage brought about by Cyclosporin caused ischaemic condition.

Key words: *Raphanas sativus*, cardioprotective, IHD, AST (GOT), LDH, uric acid Cyclosporin, antioxidant

INTRODUCTION

Raphanas sativus (Syn: Mulli) [Brassicaceae (Cruciferae)] is well reputed in the folkloric system of medicines for the treatment of gastrointestinal (GIT) and cardiovascular disorders[1] It is wildly growing plant all over the world. Many varieties are cultivated differing greatly in size, shape and color of the root on commercial scale[2]. *R. sativus* has a hot, sharp, bitter taste. It is stomachic, binding and anthelmintic. It is useful in the diseases of heart. *R. sativus* has been used in inflammations, hiccough, leprosy and cholera. It's juice relieves earaches and flowers are bechic and cholagogue[1].

R. sativus has been used as a tool in the cardiovascular disorders by the practitioners of traditional medicine[3]. The present study was, therefore, designed to validate its folkloric use. Moreover, cardiovascular diseases have been found common world over which have been caused major health problems in Pakistan. Hence, the present study was also planned to search for the effective cure of cardiac disorders, to serve the ailing humanity and to make use of the natural herbal wealth (plant source of drugs) of Pakistan. So, contribution to the improvement of economy of country by involving the industrial use of natural drugs and saving the foreign exchange presently being spent on the import of different synthetic drug (s).

MATERIALS AND METHODS

Test animals: Adult healthy albino male rabbits weighing 1.0-1.5 kg each were used in this study. The animals were housed under conditions of 23±12°C temperature, 55±15% humidity and 12 h light (7-19)[4]. They were fed according to a strict schedule with green fodder (*Medicago sativa*). Normal tap water was allowed *ad libitum*[5-7]. The animals were used in this study according to the guidelines of National Policy and in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Plant drug and extracts: *R. sativus* fruit was purchased from local market (Lahore-Pakistan), shade-dried and powdered. The fine powder was sieved out to mesh 200. The finely powdered drug (1.0 kg) was soaked in about 2.0l of distilled water and ethanol separately and extracted at room temperature. This process was repeated thrice and the respective combined filtrates were evaporated by a rotary evaporator. The dried aqueous and ethanol extracts were stored separately at -20°C till used[8,9].

Chemicals: All chemicals used were of analytical grade and obtained from E. Merck (Darmstadt, FRG), BDH Poole (England) and Sigma Chemical Co. (USA). The toxic drug was Cyclosporin E. Merck (Darmstadt, FRG).

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Induction of cardiotoxicity: The necrosis and apoptosis of cardiac tissue was produced by oral administration of Cyclosporin (30 mg kg^{-1} day^{-1})[10]. The elevated levels of Uric acid and activities of enzymes i.e. AST, LDH in serum were considered as the markers of cardiotoxicity. Animals were divided randomly into different groups of 6-8 rabbits each. The test agents were encapsulated in the gelatine shells before their oral administration[9]. All the drugs were given to the animals 8 hourly (at 6.00 am, 2.00 pm and 10.00 pm) for seven weeks[10]. The untreated control group received lactose throughout the experiment. The rabbits of treated control group were given orally Cyclosporin, 30 mg kg^{-1} day^{-1}. Three other experimental groups were treated with 0.5, 1.0 or 1.5 g kg^{-1} of powdered *R. sativus* fruit in addition to the toxic agent. Two more test groups received either the aqueous extract or ethanolic extract of *R. sativus* fruit in the doses equivalent to 1.5 g kg^{-1} of body weight and toxic agent.

Determination of serum AST (GOT) activity: Serum AST activity was measured by the method of Reitman and Frankel[11]. 0.5 ml of AST substrate in each S and B labeled test tubes was taken and the tubes were placed in water bath at 37°C. 100 μl serum in S-tube and 100 μl distilled water in B-tube were added. Then 0.5 ml 2, 4-DNPH reagent was mixed to the contents of each tube following 1.0 h incubation. After further 10 min incubation at 37°C 5.0 ml of sodium hydroxide 0.4 mol L^{-1} was added to each tube. The contents were mixed and the absorbance of sample against blank was read at 505 nm.

Determination of serum LDH activity: Enzymological activity of serum LDH was determined according to the method of Fasce[12]. To the 3.0 ml of solution containing $NADH_2$ 0.18 mmol, Pyruvate 0.6 mmol L^{-1} and Phosphate buffer 50 mmol L^{-1}, pH 7.5, 0.1 ml of fresh serum was mixed. Its mean absorbance per minute was calculated at 365 nm and LDH activity was obtained by the following formula:

$$\text{Concentration} = \Delta A_{sample}/\Delta A_{standard} \times \text{Concentration of standard}$$

Determination of serum uric acid level: Serum uric acid was oxidized by uricase to allantoin and hydrogen peroxide. Hydrogen peroxide was allowed to react with 4-aminopyrine and 3, 5-dichloro-2-hydroxybenzenesulphonate in the presence of peroxidase to form a quinoneimine dye, the concentration of which was measured at 456 nm. The serum concentration of uric acid was measured by the following formula[13]:

$$\text{Concentration} = \Delta A_{sample} / \Delta A_{standard} \times \text{Concentration of standard}$$

Determination of DPPH anti-oxidative activities: Free radical scavenging activity was determined by the method of Jeong[14]. The test substance at different concentration (equivalent to 0.5, 1.0 and 1.5 g of powdered plant) dissolved in methanol, was added to 1.5×10^{-4}M 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) methanol solution. Then the solution with the substance was shaken vigorously and kept in dark for 30 min at 25°C. The absorbance of the test solution was measured spectrophotomerically at 520 nm. L-ascorbic acid was used as a positive control (2.5-10 μg).

RESULTS AND DISCUSSION

Cyclosporin increased the serum levels of AST (69.24±2.25), LDH (241.7±2.76) and uric acid (36.85±1.39) highly significantly ($P<0.001$) in the treated control group of rabbits (Table 1). Cyclosporin has been used as an experimental tool to induce cardiac toxicity in the animals[10]. The cardiac subacute toxicity was evaluated by measuring serum enzyme activity of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)[15]. The elevated level of uric acid in the serum has also been found to be associated with subsequent morbidity and mortality in the general population among patients with congestive heart failure and other cardiac abnormalities[16].

R. sativus fruit powder attenuated the Cyclosporin induced cardiac toxicity in the dose dependent manner. The powder in the dose of 1.5 g kg^{-1} caused maximum effects in comparison to the 0.5 and 1.0 g kg^{-1} doses and it completely antagonised the cardiac effects of Cyclosporin. Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1} of body weight) also highly significantly ($P<0.001$) prevented the cardiotoxicity induced by Cyclosporin in the animals. However, ethanolic extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1} of body weight) could not caused any resistance against the effects of toxic agent (Table 1).

Cyclosporin has been found to induce the necrosis and apoptosis of cardiac tissue leading to the changes in the biochemical parameters i.e. elevation of serum levels of AST, LDH and uric acid. Cardiac enzymes like AST and LDH have been found to be increased in the plasma due to apoptosis and considered to be common characteristics of cardiotoxicity[15]. Hyperuricemia is another common finding in patients with chronic heart failure (CHF), coronary artery disease (CAD) and is associated with reduced vasodilator capacity and impaired peripheral blood flow[17,18]. Martinez *et al.*[19] has been reported that the defect in cellular oxygenation contributes to the

Table 1: Comparative effects of *Raphanus sativus* fruit and its extracts on Serum AST, LDH and uric acid in Cyclosporin treated Rabbits

Parameters	Treatments	Serum concentration/activity after interval (weeks) 0	1	2	3
Serum AST (GOT) (Ul^{-1})	Cyclosporin (30 mg kg^{-1} of body wt)	14.00±1.84	27.83±3.07**	32.50±3.89**	48.33±5.63**
	R. sativus fruit powder (0.5 g kg^{-1} of body wt) +Cyclosporin (30 mg kg^{-1} of body wt)	14.17±1.96	25.33±1.28	28.33±2.11	38.50±1.99*
	R. sativus fruit powder (1.0 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	14.83±0.79	19.83±1.49*	27.50±0.92*	30.83±1.87*
	R. sativus fruit powder (1.5 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	13.83±1.38	15.00±1.63**	16.21±1.44**	16.33±1.09**
	Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	14.08±0.97	15.96±1.63**	16.91±1.23**	17.91±2.42**
	Ethanol extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	14.51±0.73	25.25±2.15	30.21±3.14	43.21±4.51
Serum LDH (Ul^{-1})	Cyclosporin (30 mg kg^{-1} of body wt)	146.8±4.67	192.5±4.63**	226.8±3.97**	236.3±4.59**
	R. sativus fruit powder (0.5 g kg^{-1} of body wt) +Cyclosporin (30 mg kg^{-1} of body wt)	142.2±3.97	180.0±4.51	217.5±4.22	232.0±4.97
	R. sativus fruit powder (1.0 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	143.1±4.56	166.7±3.36**	181.0±3.78**	208.7±3.29**
	R. sativus fruit powder (1.5 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	140.9±5.67	148.5±4.96**	160.8±4.36**	161.0±3.56**
	Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	143.5±2.15	148.9±4.19**	162.6±3.63**	164.5±4.21**
	Ethanol extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	144.7±3.14	189.5±4.43	212.4±4.45	230.7±4.15
Serum uric acid	Cyclosporin (30 mg kg^{-1} of body wt)	25.90±1.06	32.11±1.48**	33.43±1.65**	35.11±1.15**
	R. sativus fruit powder (0.5 g kg^{-1} of body wt) +Cyclosporin (30 mg kg^{-1} of body wt)	25.46±1.07	30.15±1.04	30.97±1.13	32.10±1.43
	R. sativus fruit powder (1.0 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	25.47±1.62	29.48±1.93	29.73±1.94	30.23±1.16*
	R. sativus fruit powder (1.5 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	25.87±1.61	26.58±1.64*	26.97±1.53*	26.63±1.49**
	Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	25.91±1.06	26.36±1.61*	26.57±1.46*	26.59±1.47**
	Ethanol extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	25.88±1.08	32.09±1.65	33.04±1.84	34.44±1.53

Table 1: Continue

Parameters	Treatments	Serum concentration/activity after interval (weeks) 4	5	6	7
Serum AST (GOT) (Ul^{-1})	Cyclosporin (30 mg kg^{-1} of body wt)	68.83±3.65**	69.00±3.67**	69.20±2.41**	69.24±2.25**
	R. sativus fruit powder (0.5 g kg^{-1} of body wt) +Cyclosporin (30 mg kg^{-1} of body wt)	39.66±1.65**	42.00±1.15**	43.29±2.63**	44.00±2.75**
	R. sativus fruit powder (1.0 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	35.50±0.99**	39.50±1.18**	40.08±2.87**	41.70±3.29**
	R. sativus fruit powder (1.5 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	17.00±1.69**	17.80±1.97**	18.36±2.46**	19.57±2.74**
	Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	18.19±2.73**	18.11±2.31**	18.16±2.42**	18.11±2.31**
	Ethanol extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	64.92±5.14	65.27±4.64	65.73±2.62*	65.79±2.58*
Serum LDH (Ul^{-1})	Cyclosporin (30 mg kg^{-1} of body wt)	239.0±4.12**	240.7±3.40**	241.4±2.46**	241.7±2.76**
	R. sativus fruit powder (0.5 g kg^{-1} of body wt) +Cyclosporin (30 mg kg^{-1} of body wt)	131.5±3.58	235.3±3.88	238.6±3.18	240.7±3.71
	R. sativus fruit powder (1.0 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	211.3±3.37**	218.5±4.78**	219.1±3.14**	221.1±4.42**
	R. sativus fruit powder (1.5 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	162.3±4.62**	162.0±4.75**	162.5±4.75**	162.0±3.19**
	Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	165.9±4.42**	166.1±4.91**	166.9±4.01**	171.1±4.24**
	Ethanol extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	233.9±4.46	234.2±4.57	236.6±4.73	237.3±3.46
Serum uric acid	Cyclosporin (30 mg kg^{-1} of body wt)	35.52±1.48**	35.66±1.44**	36.25±1.23**	36.85±1.39**
	R. sativus fruit powder (0.5 g kg^{-1} of body wt) +Cyclosporin (30 mg kg^{-1} of body wt)	32.45±1.28	32.98±1.37	33.28±1.36	33.43±1.42
	R. sativus fruit powder (1.0 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	31.50±1.07*	31.68±1.06*	32.45±1.05*	32.48±1.01*

Table 1: Continue

Parameters	Treatments	Serum concentration/activity after interval (weeks) 4	5	6	7
	R. sativus fruit powder (1.5 g kg^{-1}) +Cyclosporin (30 mg kg^{-1})	26.61±1.48**	26.08±1.27**	26.29±1.40**	26.40±1.51**
	Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	26.99±1.39**	26.94±1.56**	27.33±1.60**	27.34±1.46**
	Ethanol extract of *R. sativus* fruit powder (Eq. to 1.5 g kg^{-1})+Cyclosporin (30 mg kg^{-1})	35.17±1.54	35.06±1.45	36.12±2.43	36.47±2.58

Cyclosporin: significant from 0.0 hour (Normal) * P<0.05; ** P<0.001. Test drugs: significant from treated control (Cyclosporin) * P<0.05; ** P<0.001. All the other values are N.S. (P>0.05) from treated control (Cyclosporin). Mean±S.E.M = Mean values±Standard error of means of six experiments

Table 2: Free radical scavenging activity of *Raphanus sativus* fruit powder and its aqueous fraction

Treatments	Inhibition (%)	IC_{50}
Ascorbic acid (2.5 μg ml^{-1})	8.2	6.4 μg ml^{-1}
Ascorbic acid (5 μg ml^{-1})	17.3	
Ascorbic acid (10 μg ml^{-1})	75.9	
R. sativus fruit powder (0.5 g ml^{-1})	17.4	1.2 g ml^{-1}
R. sativus fruit powder (1.0 g ml^{-1})	26.3	
R. sativus fruit powder (1.5 g ml^{-1})	46.1	
Aqueous extract of *R. sativus* fruit powder (Eq. to 0.5 g ml^{-1})	14.2	1.3 g ml^{-1}
Aqueous extract of *R. sativus* fruit powder (Eq. to 1.0 g ml^{-1})	23.1	
Aqueous extract of *R. sativus* fruit powder (Eq. to 1.5 g ml^{-1})	45.7	

elevation of plasma uric acid levels in patients with chronic cardiac failure. Serum uric acid is strongly related to circulating markers of inflammation in patients with chronic heart failure. This is consistent with a role for increased xanthine oxidase (XO) activity in the inflammatory response in patients with chronic heart failure[20]. In hyperuricemic CHF patients, XO inhibition with allopurinol improves peripheral vasodilator capacity and blood flow both locally and systemically. It has been further suggested that the causal link of this association is increased xanthine oxidase (XO)-derived oxygen free radical production and endothelial dysfunction[19]. The chronic cardiotoxicity may result from the summation of multiple biochemical pathways of cellular damage, which ultimately yields to disruption of myocardiocyte integrity and loss of cardiac function. Nitric oxide (NO) is a key molecule involved in the pathophysiology of heart; dysregulation of activity of NO synthases (NOSs) and of NO metabolism seems to be a common feature in various cardiac diseases. Overproduction of ROS and NO yields to reactive nitrogen species, particularly, the powerful oxidant molecule peroxynitrite (ONOO-), which may produce the marked cardiotoxicity[21,22]. Hence, uric acid may serve as an additional marker of free radical reactions in patients with acute myocardial infarction and acute coronary insufficiency[23] .

R. sativus and its aqueous extract showed free radical-scavenging effect in a dose dependent manner on DPPH assay (Table 2). This effect was highly significant (P<0.001) with the dose equivalent to 1.5 g kg^{-1} of the aqueous extract of powdered *R. sativus* fruit and powder. The IC_{50} of the substance, its aqueous extract and ascorbic acid are 1.2 , 1.3 g ml^{-1} (equivalent to plant drug) and 6.4 μg ml^{-1}, respectively (Table 2). Therefore, it can be speculated that *R. sativus* and its aqueous extract strengthen the antioxidant defense system to resist the free radical induced damage brought about by Cyclosporin induced ischaemic condition. The data is in accordance to the findings of Chattopadhyay *et al.*[24].

In conclusion, the reported results have validated the folkloric use of the drug tested for use in the therapy of cardiac diseases. In particular, the present studies have pointed out possible cardioprotective effects of the *R. sativus* fruit and its aqueous extract. Nevertheless, detailed chemical studies followed by pharmacological investigations and toxicity evaluations are still required to isolate the pure active principle(s) of the *R. sativus* fruit and to elucidate their mode(s) of cardioprotective actions studies are also needed.

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