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ETHANOLIC *BRASSICA OLERACEAE ITALICA* EXTRACT PRETREATMENT ALLEVIATES ISOPROTERENOL INDUCED OXIDATIVE MYOCARDIAL NECROSIS IN RATS.

Gowri S*, Suganthi V, Dr.Gurusamy K

PG and Research Department of Biochemistry, Dr.N.G.P Arts and Science College, Coimbatore, India.

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***Correspondence for Author:**

Gowri S,

PG and Research Department of Biochemistry, Dr.N.G.P Arts and Science College, Coimbatore, India

akshayagowri@gmail.com,

ABSTRACT

Ethanolic *Brassica oleraceae italica* extract (200 mg/kg) pretreatment for 30 days in Isoproterenol (ISO) treated rats significantly decreased the levels of myocardial marker enzymes (lactate dehydrogenase, creatine kinase, Transaminases), uric acid and increased myocardial lipid peroxides. Histological examination of rat's heart section confirmed myocardial injury with ISO administration and near normal pattern with ethanolic *Brassica oleraceae italica* extract pretreatment. The results of the present study for the first time provide clear evidence that the ethanolic *Brassica oleraceae italica* extract pretreatment enhances the antioxidant defense against ISO induced oxidative myocardial injury in rats and exhibit cardio protective property.

Key words: *Brassica oleraceae italic*, Isoproterenol (ISO), Myocardial

INTRODUCTION

Myocardial infarction (MI) the most dreaded sequel among ischemic heart disease is invariably followed by several biochemical alterations such as lipid peroxidation, free radical damage, hyperglycaemia, hyperlipidemia etc., and leading to qualitative and quantitative alteration of myocardium [1].

Oxygen free radicals are implicated as indicators of tissue injury in cardiovascular pathology [2].Free radical generation and lipid peroxidation could be involved in isoproterenol (ISO) - induced cardiac damage [3].

ISO induced myocardial infarction increases lysosomal hydrolase activities, which may be responsible for tissue damage and infracted heart [4] and also causes alterations in the fragility of lysosomal membrane of the heart [5].

Despite considerable progress in the management of myocardial infarction by synthetic drugs, the search for indigenous cardio protective agents still continue. Some plant products have also been demonstrated to cause augmentation of myocardial antioxidants [6, 7].

Brassica oleraceae italica (BOI) commonly called as Broccoli belongs to the family of Brassicaceae. Many plants in the Brassicaceae family are important vegetables worldwide. Broccoli is believed to be the first Cole crops to evolve from the wild species of kale or cabbage and was cultivated by the Romans.

The Americans cancer society proved that the mortality rate of laboratory animals exposed to radiation could be reduced significantly by feeding of broccoli. Broccoli is exceptionally rich source of inducers of enzymes that protect against chemical carcinogenesis [8]. Broccoli has excellent antioxidant potential and gives protection against radical induced oxidative damage [9].The liphophilic and hydrophilic extract of broccoli has oxygen radical absorbance capacity and protects DNA from oxidative damage [10].

Experimental evidence on biochemical role of broccoli extract in myocardial damage is lacking and the present study has been designed to find out whether oral pretreatment of ethanolic Brassica oleraceae italica extract could exert any protective action against ISO induced myocardial injury.

In this context, an attempt has been made to elucidate the maintenance of myocardial integrity in presence and absence of BOI on ISO induced cardiac damage with reference to biochemical cardiac markers and histology.

MATERIALS AND METHODS

Collection of the Plant Material

The flower clusters of *Brassica oleraceae italica* were collected from Coimbatore district of Tamilnadu.

Extraction and Preparation of Extract

The plant parts were washed, shade dried and powdered. The powdered materials were extracted with ethanol using a soxhlet extraction apparatus. The ethanolic extract was concentrated and dried under reduced pressure. It was dissolved in distilled water and administrated orally with intragastric feeding tube.

Selection of Animals

Healthy adult male wistar albino rats weighing about 180- 230 g were obtained from Sri Venkateshwara enterprises, Bangalore. The animals were housed in large poly propylene spacious cages, maintained in controlled temperature, humidity and 24 hours light/ dark cycles. They were fed with standard pelleted diet obtained from Hindustan lever limited, Bangalore and water adlibitium. The experimental animals were acclimatized to laboratory conditions for 10 days. Animals were maintained as per the principles and guidelines of the ethical committee for animal care of KMCH college of pharmacy in accordance with the Indian national law of animal care and use

Experimental Induction of Cardiac Injury

Cardiac damage was induced in experimental rats by subcutaneous injection of isoproterenol hydrochloride dissolved in normal saline at a dose of 85 mg / kg body weight.

Experimental Design of Animals:

The rats were divided into 4 groups of six animals each as given in Table 1.

Group	Experimental Design
I	Control rats – received normal pelleted diet
II	Toxic rats- isoproterenol hydrochloride was injected (85 mg / kg body weight) as single dose on 29^{th} and 30^{th} day, at an interval of 24 hours by subcutaneous injection.
III	*Brassica oleraceae italica* treated groups by oral administration 100 mg/ day once daily for 30 days.
IV	*Brassica oleraceae italica* treated groups by oral administration 250 mg/ day once daily for 30 days

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Collection of heart and blood sample

At the end of the experimental period, i.e., 24 hours after last injection of isoproterenol hydrochloride, the animals were killed by cervical decapitation. The blood was collected in ice cold container without any anticoagulant and the serum was separated. Immediately after the sacrifice, the rats were dissected; heart was removed and washed with ice cold saline. One portion of the tissue was fixed in 10% formalin- saline for histopathological observation.

Estimation of biochemical parameters

The specific marker enzymes for myocardial infarction viz, Lactate dehyrogenase (LDH) [11], Creatine kinase (CK) [12] , Aspartate transaminase (AST)[13] and Alanine transaminase (ALT) [13] were measured in serum. The level of uricacid [14] present in serum also measured.

Histopathological observation

Myocardial tissues were fixed in 10% formalin, routinely processed and embedded in paraffin wax. Paraffin section were cut on glass slides and stained with hematoxylin and eosin (H&E), and examined under a light microscope by a pathologist blinded to the groups studied.

Statistical analysis

Results were expressed as Mean± SD of 6 animals. One way analysis of variance was employed for the determination of variations in a set of data. Differences among means were analyzed by least significant difference procedure.

RESULTS AND DISCUSSION

Serum LDH, CK, and Transaminases showed significant increase in their activity in group II animals when compared to groupI animals (Table II). BOI pretreated rats restored the activities of these enzymes to near normal.

Serum uric acid level was fund to be significantly increased in ISO treated rats and it was significantly reduced in BOI pretreated rats when compared to group II animals (Figure I).

Serum levels of LDH, CK, and Transaminases are the diagnostic indicators of myocardial infarction [15, 16].

An increase in the activities of marker enzymes in serum could be due to the leakage of enzymes from heart as result of isoproterenol induced necrosis [17] and the amount of enzymes appear in serum s proportional to the number necrotic cells [18].

Increase in serum uric acid in experimental animals could be due to excessive degradation of purine nucleotides and proteolysis [19].

Reduced necrotic changes in BOI treated animals could be the reason for the decreased activities of the enzymes in group IV animals.

Exogenous administration of antioxidants could provide protection from myocardial necrosis by free radical scavenging activity or by antioxidant activity [20, 21].

Pharmacological argumentation of endogenous myocardial antioxidants has been identified as a promising therapeutic approach in diseases associated with increased oxidative stress [22, 23].

Induction of phase II detoxification enzymes is a powerful strategy for achieving protection against carcinogenesis, mutagenesis and other forms of toxicity of electrophiles and reactive oxygen species. BOI contains very large quantity of isothiocyanates which are very potent inducers of phase II enzymes [24].

Glucoraphanin, rich broccoli help the body to disarm free radical by boosting the body's own antioxidant defense system by increasing levels of glutathione, an antioxidant produced by the body that serves as an essential component in glutathione reductase and glutathione peroxidase and some of the livers most important detoxification enzymes [25].

TABLE – II Effect of *Brassica oleraceae* flower extract on serum AST, ALT, LDH and CK level of ISO treated rats

Groups	AST (IU/L)	ALT (I U/L)	**LDH (1U /L)**	**CK (1U /L)**
Vehicle control Water for injection	8.92 ± 0.08	9.7 ± 0.4	216.7 ± 18.9	39.0 ± 2.81
Negative Control ISO 85 mg/kg	16.6 ± 1.2 a**	20.1 ± 1.6 a**	408.5 ± 34.6 a**	142 ± 9.7 a**
Test group 1 BOI 100 mg/kg	11.6 ± 1.02 b**	16.2 ± 1.1 b**	250.7 ± 15.3 b**	78.4 ± 9.7 b**

+ ISO 85 mg/kg				
Test group 2 BOI 100 mg/kg + ISO 85 mg/kg	7.91 ± 0.06 c**	11.3 ± 0.8 c**	226.8 ± 21.7 c**	56.5 ± 3.7 c**

Values are expressed as mean ± SD of six animals

Group comparison a - Group I Vs Group II

b - Group II Vs Group III

c - Group II Vs Group IV

Statistical comparison * - P□ 0.05

** - P □ 0.01

FIGURE – I Effect of *Brassica oleraceae* flower extract on serum Uric acid level of ISO treated rats

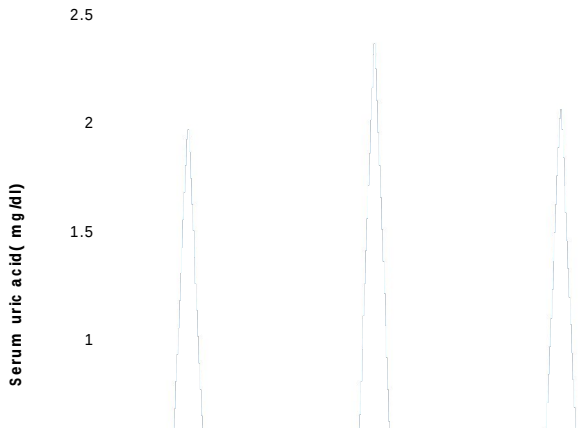


Plate – I Normal group rat's heart section, showing normal myocardial fibres (10x)

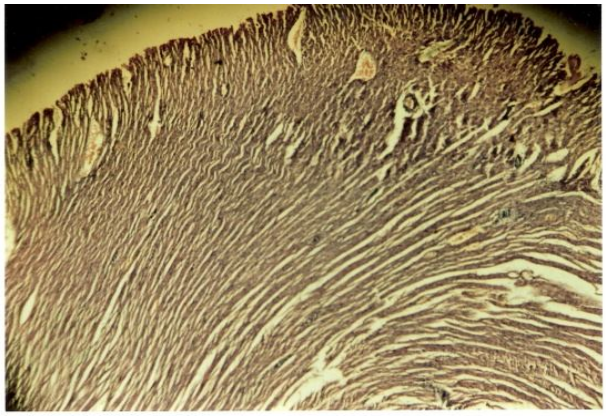


Plate – II Pathogenic control group rat's heart section treated with IPH (85mg/kg body weight, S.C) showing marked inflammatory infiltrate with odema (10x)

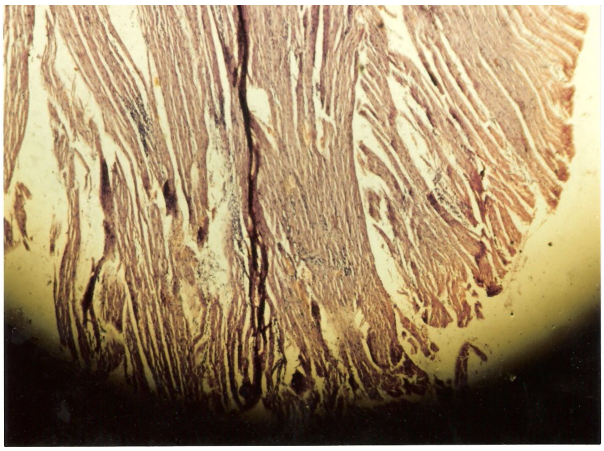


Plate – III

Heart of rat treated with IPH and *Brassica oleraceae* flowers showing normal myocardial fibres (100mg dosage) (10x)

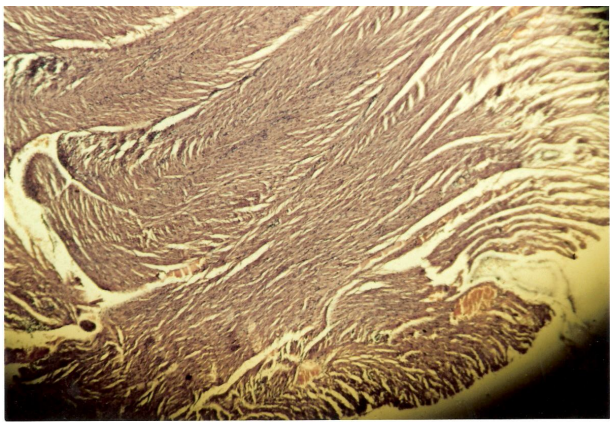
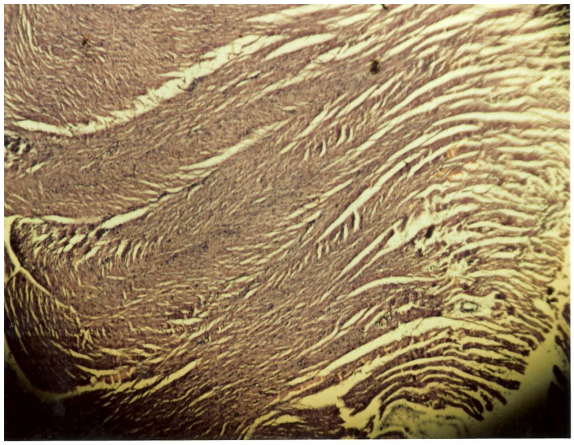


Plate – IV Heart of rat treated with IPH and *Brassica oleraceae* flowers showing efficiently preserved myocardial fibres (200 mg dosage (10x)



So the present study can be concluded that regular consumption of BOI in diet may provide significant protection against myocardial infarction due to its high glucosinolates, flavanoids and nutrition content.

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