

EVALUATION OF ANTI-HYPERTROPHIC POTENTIAL OF *CAMELLIA SINENSIS* IN ISOPROTERENOL INDUCED CARDIAC HYPERTROPHY

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ABSTRACT

Objective: *Camellia sinensis* (*C. sinensis* family-Theaceae) has potent antioxidant activity used in the treatment of cardiovascular disease. The present study evaluates the cardioprotective (anti-hypertrophic) effect of aqueous extract of *C. sinensis* in isoproterenol (ISO) induced cardiac hypertrophic rats.

Methods: The beneficial effect of the green tea extract was examined by the administration of the aqueous extract of the leaves of *C. sinensis* (100 mg/kg b.w., oral, 7 d) in ISO (10 mg/kg b.w., subcutaneous, 7 d) induced cardiac hypertrophic rats with reference to the standard drug, losartan (50 mg/kg b.w., oral, 7 d) followed by biochemical estimations of glucose, protein, cholesterol, cardiac marker enzymes namely serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and lactate dehydrogenase (LDH) in serum and heart tissues thus collected at the end of 7 d.

Results: The biochemical assays revealed significantly ($P < 0.05$) increased glucose, protein, cholesterol, cardiac marker enzymes namely serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) lactate dehydrogenase (LDH) and significantly ($P < 0.05$) decreased in ISO induced cardiac hypertrophic rats that were reciprocated by the effect of plant extract.

Conclusion: Thus, this study showed that the aqueous leaf extract of *C. sinensis* possesses potent effect against cardiac hypertrophy. This potential is hypothesized to be due to the phytochemical, Catechin present in the plant that requires further isolation and characterization with respect to anti-hypertrophic therapeutics.

Keywords: *Camellia sinensis*, Isoproterenol, Cardiac hypertrophy

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INTRODUCTION

Cardiovascular disease (CVD) is one of the leading causes of death globally, more people die annually from CVDs than from any other cause. An estimated, 17.7 million people died from cardiovascular diseases in 2015, representing 31% of all global deaths. Out of these deaths, 7.4 million were due to coronary heart disease and 6.7 million were due to Stroke. Over three quarters of CVD deaths take place in low and middle-income countries. Among 17 million premature deaths (under the age of 70) due to non-communicable diseases in 2015, 37% are caused by cardiovascular diseases and 82% are in low and middle-income countries.

"Cardiac hypertrophy is the abnormal enlargement or thickening of the heart muscle resulting from increases in cardiomyocyte size and changes in other heart muscle components, such as extracellular matrix". Hypertrophic growth accompanies many forms of cardiovascular diseases such as hypertension, heart failure and valvular disease [1]. It is a response of myocardium to various physiologic and pathologic stimuli that causes the heart to work harder under condition of increased workload [2].

Administration of several medicinal plants have restored the activities of certain serum cardiac enzymes when compared to normal control groups [3]. The active phytoconstituents of various plant species are isolated for the direct use of drugs, lead compounds or pharmacological agents.

Green tea (*Camellia sinensis* or *C. sinensis*; family-Theaceae) is made from more mature tea leaves, and they may be withered prior to steaming or firing. Although they are also rich in catechins, green tea may have catechins profiles different from other tea with slightly higher levels of oxidation products [4]. Tea catechins can act as antioxidants by donation of a hydrogen atom, as an acceptor of free radicals, interrupting chain oxidation reactions, or by chelating

metals [5]. The active ingredients present in the tea leaves is polyphenol and it's the important biological compound [6]. Furthermore, this plant has several medicinal values attributed to high polyphenol contents having tremendous impact on health [7]. Many of the studies of polyphenols and catechins reports mechanisms with protection against degenerative diseases [8, 9]. Green tea has hepatoprotective activity [10], antidiabetic effect [11], cardioprotective activity [12], and antimicrobial activity [13]. Various formulations of green tea are prepared that can be beneficial via its antioxidant properties against cellular toxicities [14, 15]. This study anticipates in evaluating the anti-hypertrophic potential of *C. sinensis* by ameliorating the effects of isoproterenol induced cardiac hypertrophy.

MATERIALS AND METHODS

Chemicals

All the chemicals were purchased as analytical grade from Hi-Media Laboratories, India. Isoproterenol was purchased as Isoprenaline hydrochloride from Sigma-Aldrich. The standard drug losartan was purchased commercially as losarpen (25 mg) tablets from local pharmacy, India. The glucose and cholesterol estimation kits were purchased from Arkray Healthcare Pvt, Ltd., India. The triglyceride kit was purchased from Ensure Biotech Pvt, Ltd., India. The SGOT and SGPT kits were purchased from Microlyn Healthcare Pvt, Ltd., India.

Plant collection and extraction

The whole plant of *Camellia sinensis* were collected during the month of December, 2017 from the local areas (folklore shops) of Coimbatore district, Tamil Nadu, India. The plant was dried in shade at room temperature. The dried whole plant was submitted and authenticated (No. BSI/SRC/5/23/2017/Tech/1955) at Botanical Survey of India, Southern Regional Centre, Coimbatore, India. The leaves of *C. sinensis* were shade dried and powdered in a mixer

grinder. The aqueous extract of *C. sinensis* whole plant was prepared with plant powder: water (1:3) cold macerated for 48 h, filtered and allowed to dry under controlled temperature to yield the dried leaf aqueous extract [16].

Procurement of animals

Young male Albino rats of Wistar strain (200g) procured and the Ethical clearance for handling of experimental animals was obtained from the Institutional Animal Ethics Committee (IAEC) constituted for the purpose and care of laboratory animals as per the guidance of the Committee for the Purpose of Control and Supervision of

Experiments on Animals (CPCSEA), Ministry of Social justice and empowerment, Government of India (CPCSEA/No 387/2018/IAEC) at the PSG Institute of Medical Sciences and Research (PSG IMSandR), Coimbatore.

Experimental design

The experimental rats were divided into 4 groups of 6 animals in each group. Cardiac hypertrophy was induced to Albino Wistar rats using isoproterenol and simultaneous treatment using the plant extract and the reference drug, losartan was carried out as shown in table 1.

Table 1: Experimental groups of induction and treatment of cardiac hypertrophy

Groups	Experimental animals
Group I	Normal control rats
Group II	Isoproterenol (10 mg/kg b.w., s. c., 7 d)[17]
Group III	Isoproterenol+Losartan (50 mg/kg b.w., oral., 7 d)[18]
Group IV	Isoproterenol+aqueous leaf extract of <i>C. sinensis</i> (100 mg/kg b.w., oral., 7 d)[19]

After the end of the experimental treatment period (7 d), the animals were sacrificed under mild chloroform anesthesia. Blood was collected by cardiac puncture and the serum was separated by centrifugation at 5000 rpm for 10 min. The heart tissue was excised immediately and thoroughly washed in saline before use. A 10% homogenate of the washed animal tissue was prepared using 0.1 M Phosphate buffer (pH 7.4) in potter homogenizer fitted with a teflon plunger running at 6000 rpm for 3 min. The thus prepared homogenate was used for various biochemical assays.

Biochemical estimation

Serum glucose was assayed using glucose oxidase method (Autospan Liquid Gold Glucose Kit), serum total protein by Lowry's method [20], serum albumin using Bromo cresol green end point assay method (Autospan), estimation of serum total cholesterol using POD-PAP enzymatic end point assay (Autospan), estimations of SGOT and SGPT activities by modified IFCC method (Microlyn) and determination of LDH activity by optimized kinetic assay method (Autospan) followed by estimation of superoxide dismutase (SOD)[21], catalase [22] and glutathione peroxidase (GPx) [23].

Statistical analysis

Data obtained was expressed as mean±SD. Statistical analysis was performed by using the method of distribution statistics (Standard descriptive analysis) and analysis of mean (Student's 't' test) using R-Statistical Computing and Graphical Tools (formerly AT and T Lucent technology). A probability of P<0.05 was considered significant.

RESULTS AND DISCUSSION

Effect of ISO and plant extract on the hypertrophic indices

The gross examination of the heart showed an increase in the heart size in the isoproterenol group when compared to the normal group fig. 1. On anatomical observation no, remarkable cavity effusions in hypertrophic groups were observed. The heart weight/body weight ratio (HW/BW ratio) as evident from table 2 was found to be increased by 78.72% in the isoproterenol administered rats (group I) when compared to control rats (group II), demonstrating an increase in the heart size. However, Losartan pretreated rats (group III) showed a 22.78% reduction in the HW/BW ratio when compared to isoproterenol administered rats. Similar to other studies attenuation of the progression of cardiac hypertrophy is indicated with reduced HW/BW ratio in plant extract administered groups [24].

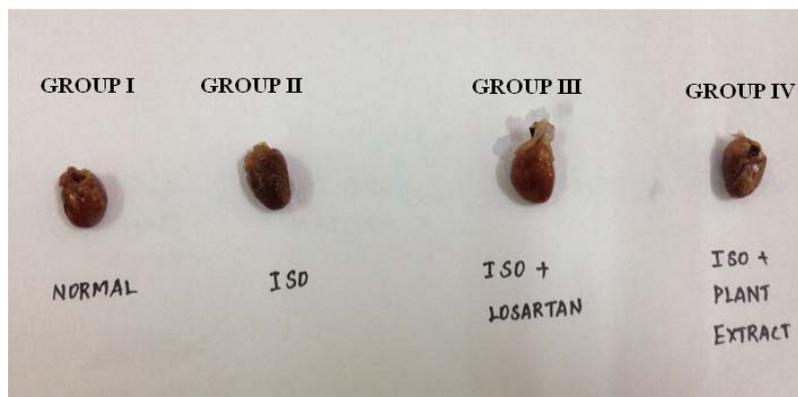


Fig. 1: Examination of the heart size in isoproterenol induced experimental rats and *C. sinensis* treated rats

Table 2: Effect of *C. sinensis* on the HW/BW ratio in the control and experimental rats

Groups	Heart weight (HW) (mg)	Body weight (BW) (g)	HW/BW
Group I	410±10	170±12.92	2.41±0.77
Group II	475±4.58 ^{a*}	187±16.70 ^{a*}	2.54±0.27 ^{a*}
Group III	442±2.5 ^{b*}	190±17.58 ^{b*}	2.33±0.14 ^{b*}
Group IV	456±0.6 ^{c*}	190±12.59 ^{c*}	2.4±0.05 ^{c*}

Table values are expressed by mean±SD of 6 samples per group. Group comparison: a-Normal (I) Vs ISO (II); b-ISO (II) Vs Drug (III); c-ISO (II) Vs Plant extract (IV). Statistical significance (P<0.05) indicated by*

Effect of ISO and plant extract on glucose and total protein in heart tissue and serum

Amelioration of cardiac hypertrophy is indicated by significant decrease in the elevated levels of glucose, protein, total cholesterol and significant increase in reduced HDL cholesterol levels [25]. In

isoproterenol induced cardiac hypertrophic rats, the glucose, total protein and total cholesterol levels in serum and heart tissue were significantly ($P < 0.05$) increased considerably with that of the normal group. After oral administration of the aqueous leaf extract of *C. sinensis* and losartan the glucose levels were restored in experimental animals similar to that of normal control as shown in table 3 and 4.

Table 3: Effect on serum glucose, total protein and total cholesterol

Groups	Serum glucose (mg/g)	Serum protein (mg/g)	Serum cholesterol (mg/g)
Group I	109.37±0.26	24±0.56	28.76±0.36
Group II	141.36±0.27a*	38±0a*	65.58±1.007a*
Group III	113.41±0.28b*	21±1b*	32.09±0.07b*
Group IV	119.44±0.325c*	16±0.6c*	41.11±0.10c*

Table values are expressed by mean±SD of 6 samples per group. Group comparison: a-Normal (I) Vs ISO (II); b-ISO (II) Vs Drug (III); c-ISO (II) Vs Plant extract (IV). Statistical significance ($P < 0.05$) indicated by*

Table 4: Effect on glucose, protein and cholesterol in heart tissue

Groups	Tissue glucose (mg/g)	Tissue protein (mg/g)	Tissue cholesterol (mg/g)	Serum HDL (IU/l)
Group I	108.52±0.26	7±0.56	32.56±0.36	19.50±0.036
Group II	151.7±0.27a*	21±0a*	68.46±1.007a*	65.58±1.007a*
Group III	110.14±0.28b*	4±1b*	32.92±0.07b*	32.09±0.075b*
Group IV	113.44±0.325c*	4±0.6c*	36.38±0.10c*	41.11±0.10c*

Table values are expressed by mean±SD of 6 samples per group. Group comparison: a-Normal (I) Vs ISO (II); b-ISO (II) Vs Drug (III); c-ISO (II) Vs Plant extract (IV). Statistical significance ($P < 0.05$) indicated by*

Effect of ISO and plant extract on cardiac marker enzymes

Elevated cardiac enzymes are an indication of cardiac hypertrophy which is a key risk factor for myocardial infarction [26]. Administration of isoproterenol led to a significant ($P < 0.05$) increase

in levels of SGOT, SGPT and LDH levels in cardiac hypertrophic rats when compared with normal control rats. After treatment with standard Losartan drug in cardiac hypertrophic rats and plant extract to hypertrophic rats for a period of 7 d there was a significant decrease in the levels of SGOT, SGPT and LDH as shown in table 5 and 6.

Table 5: Levels of SGOT, SGPT and LDH in serum of control and experimental rats

Groups	Serum SGOT (IU/l)	Serum SGPT (IU/l)	Serum LDH (IU/l)
Groups I	0.72±0.009	0.09±0.01	1.08±0.017
Groups II	0.795±0.03a	0.68±0.06a*	1.35±0.005a*
Groups III	0.707±0.036b*	0.11±0.004b*	1.183±0.12b*
Groups IV	0.682±0.057c*	0.08±0.02c*	1.064±0.15c*

Table values are expressed by mean±SD of 6 samples per group. Group comparison: a-Normal (I) Vs ISO (II); b-ISO (II) Vs Drug (III); c-ISO (II) Vs Plant extract (IV). Statistical significance ($P < 0.05$) indicated by*

Table 6: Levels of SGOT, SGPT and LDH in tissue of control and experimental rats

Groups	Tissue SGOT (IU/l)	Tissue SGPT (IU/l)	Tissue LDH (IU/l)	Tissue HDL (IU/l)
Group I	0.79±0.002	0.19±0.01	1.006±0.002	32.56±1.28
Group II	0.80±0.005a*	0.34±0.001a*	1.07±0.009a*	68.46±1.80a*
Group III	0.78±0.001b*	0.07±0.03b*	1.35±0.00b*	32.92±1.05b*
Group IV	0.697±0.123c*	0.2±0.04c*	1.06±0.003c*	36.38±0.28c*

Table values are expressed by mean±SD of 6 samples per group. Group comparison: a-Normal (I) Vs ISO (II); b-ISO (II) Vs Drug (III); c-ISO (II) Vs Plant extract (IV). Statistical significance ($P < 0.05$) indicated by*

In the present study, treatment with *C. sinensis* leaf extracts which effectively reduced both serum and tissue SGOT, SGPT and LDH levels in cardiac hypertrophic rats suggesting that the extracts of experimental plant prevent cardiac injury associated with cardiac hypertrophy.

Flavonoids, phenols, carotenoids and glycosides are the major phytoconstituent present in the *C. sinensis*. Catechin is the flavonoid compound reported to have cardioprotective activity.

Effect of ISO and plant extract on enzymic antioxidants

Regulated antioxidant system is essential for successful therapy to treat cardiac hypertrophy [27]. In isoproterenol induced cardiac hypertrophic rats, the antioxidant enzymes catalase, SOD, GPx were significantly ($P < 0.05$) decreased considerably with that of the normal group. Oral administration of the aqueous extract restored the above antioxidants in experimental animals similar to that of normal control.

Table 7: Levels of enzymatic antioxidants in serum of control and experimental rats

Groups	Catalase (IU/l)	SOD (IU/l)	GPx (IU/l)
Group I	40.45±0.11	54.74±0.13	29.67±0.1
Group II	12.11±0.11a*	30.9±0.06a*	13.62±1.154a*
Group III	23.12±0.06b*	43.0±0.19b*	28.60±0.152b*
Group IV	20.04±0.04c*	46.01±0.1c*	19.34±0.02c*

Table values are expressed by mean±SD of 6 samples per group. Group comparison: a–Normal (I) Vs ISO (II); b–ISO (II) Vs Drug (III); c–ISO (II) Vs Plant extract (IV). Statistical significance (P<0.05) indicated by*

Table 8: Levels of enzymatic antioxidants in heart of control and experimental rats

Groups	Catalase (IU/l)	SOD (IU/l)	GPx (IU/l)
Group I	40.45±0.11	54.74±0.13	29.67±0.1
Group II	12.11±0.11a*	30.9±0.06a*	13.62±1.154a*
Group III	23.12±0.06b*	43.0±0.19b*	28.60±0.152b*
Group IV	20.04±0.04c*	46.01±0.1c*	19.34±0.02c*

Table values are expressed by mean±SD of 6 samples per group. Group comparison: a–Normal (I) Vs ISO (II); b–ISO (II) Vs Drug (III); c–ISO (II) Vs Plant extract (IV). Statistical significance (P<0.05) indicated by*

CONCLUSION

The present work implies that the aqueous extract of *C. sinensis* leaves restored the serum and heart tissue levels of glucose, protein, cholesterol, cardiac markers enzymes (SGOT, SGPT and LDH) and enzymatic antioxidants (SOD, Catalase and GPx) which may be due to the phytochemical, Catechin present in the plant. Further studies may be taken up for the isolation and characterization of catechin and also other bioactive antioxidant and cardioprotective compounds which may exert potent anti-hypertrophic effects.

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AUTHORS CONTRIBUTIONS

All authors contributed equally in designing the work, performing and interpreting the experiments followed by manuscript preparation.

RESPONSE TO COMMENTS

We thank the reviewers and the editorial board for their valuable comments and we hereby declare that the applicable revisions were carried out cautiously in the article order to meet the scientific needs of the study.

CONFLICT OF INTERESTS

There is no conflict of interest among the authors

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