EVALUATION OF ANTI-HYPERTROPHIC POTENTIAL OF CAMELLIA SINENSIS IN ISOPROTERENOL INDUCED 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 nature 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 report mechanisms with protection against degenerative diseases [8, 9]. Green tea has hepatoprotective activity [10], anti-diabetic 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 Micromyl 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
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.

| 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 *C. sinensis* (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].

| 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±4.59              | 187±16.70            | 2.54±0.27 |
| Group III | 442±2.59             | 190±17.58           | 2.33±0.14 |
| Group IV | 456±0.6               | 190±12.59           | 2.40±0.05 |

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.

| 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 | 149.36±0.27a*        | 38±0.9a              | 65.58±1.007a*            |
| Group III| 113.41±0.28b*        | 21±1b*               | 32.09±0.07b*             |
| Group IV | 119.44±0.32c         | 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 of ISO and plant extract on enzymic antioxidants

| Groups   | Tissue glucose (mg/g) | Tissue protein (mg/g) | Tissue cholesterol (mg/g) | Serum HDL (IU/l) |
|----------|----------------------|----------------------|--------------------------|------------------|
| Group I  | 108.5±0.26           | 7±0.56               | 32.56±0.36               | 19.50±0.036      |
| Group II | 151.7±0.27a*         | 21±1a*               | 68.46±1.007a*            | 65.58±1.007a*    |
| Group III| 111.0±0.28b*         | 4±1b*                | 32.92±0.07b*             | 32.09±0.075b*    |
| Group IV | 113.4±0.32c         | 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 enzymic antioxidants

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±0.17       |
| Groups II| 0.795±0.03a      | 0.68±0.06a       | 1.35±0.005a     |
| Groups III| 0.707±0.036b*   | 0.11±0.004b*     | 1.18±0.12b*     |
| Groups IV| 0.682±0.057c*   | 0.08±0.02c*      | 1.06±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) |
|----------|-------------------|-------------------|-----------------|
| Groups I | 0.79±0.002        | 0.19±0.01         | 1.00±0.002      |
| Groups II| 0.80±0.005a*     | 0.34±0.001a*      | 1.07±0.009a*    |
| Groups III| 0.78±0.001b*    | 0.07±0.03b*       | 1.35±0.00b*     |
| Groups IV| 0.697±0.123c*    | 0.2±0.04c*        | 1.06±0.003c*    |

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 enzyme 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.

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.
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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REFERENCES

1. Frey N, Katus HA, Olson EN, Hill JA. Hypertrophy of the heart: a new therapeutic target. Circulation 2004;109:1580-9.
2. Carreno JR, Apablaza F, Ocaranza MP, Jalil JE. Cardiac hypertrophy: molecular and cellular events. Rev ESP Cardiol 2006;59:473-86.
3. Bopanna KN, Kannan J, Godgl J, Bakaran R, Rathod SP. Antidiabetic and Antihyperlipidemic effects of Neem seed kernel powder on alloxan diabetic rabbits. Indian J Pharmacol 1997;29:162-72.
4. Chu DC, Juneja LR. General chemical composition of green tea and its infusion. In: Juneja LR, Chu DC, Kim M, [Eds] Chemistry and application of green tea. CRC press, Boca Raton; 1997, p. 13-22.
5. Gramza A, Korczak J. Tea constituents (Camellia sinensis L.) as antioxidants in lipid systems. Trends Food Sci Tech 2005;16:351-8.
6. Hara Y. Green tea: health benefits and applications. Bola Raton, FL CRL Press; Taylor and Francis group; 2001. p. 252.
7. Cabrera C, Artacho R, Gimenez R. Beneficial effects of green tea: a review. J America College Nutr 2006;25:79-99.
8. Nie G, Cao Y, Zhao B. Protective effects of green tea polyphenols and their major components, (−) Epigallocatechin-3-gallate (EGCG), on 6-hydroxypodamine-induced in PC 12 cells. Redox Rep 2002;7:171-7.
9. Huang Q, Wu LJ, Tashiro S, Gao HY, Onodera S, Ikejima T. (+) catechin, an ingredient of green tea, protects marine microglia from Oxidative stress-induced DNA damage and cell cycle arrest. J Pharmacol Sci 2005;98:16-24.
10. Schuppman DJ, Jia B,rikhaus EHG, Hahn. Herbal products for liver disease: a therapeutic challenge for the new millennium. Hepatology 1999;30:1099-104.
11. Tsuneki H, Ishizuk M, Terasawa M, Jin-Bi W, Sasaoka T, Kimura I. Effect of green tea on blood glucose levels and serum proteomic patterns (db/db) mice and on glucose metabolism in healthy humans. BMC Pharmacol2004;26:18-28.
12. Chisaka T, Matsuda H, Kubomura Y, Mochizuki M, Yamahara J, Fujimura H. The effect of crude drugs on experimental hypercholesterolemia: mode of action by (-) epigallocatechin gallate in tea leaves. Chem Pharm Bull 1998;36:227-33.
13. Arakawa H, Maeda M, Shimamura T. Role of hydrogen peroxide in bacterial cell death. Biochem Bioph Bull 2004;27:277-81.
14. Anwar E, Utami TD, Ramadon D. Transfersomal gel containing green tea (Camella sinensis L. Kunte) leaves extract: increasing in vitro penetration. Asian J Pharm Clin Res 2017;10:1-5.
15. Murale B, Upadhyaya UM, Goyal RK. Effect of chronic treatment with Eucocenstoma littorale in non-insulin dependent diabetic (NIDDM) rats. Ethiopharmacol2002;81:199-204.
16. Zhang S, Tang F, Yang Y, Lu M, Luan A, Zhang J, et al. Astragalolide IV protects against isoproterenol-induced cardiac hypertrophy by regulating NF-κB/PGC-1α signaling mediated energy metabolism. Plosone 2015;1:1-8. https://doi.org/10.1371/journal.pone.018759.
17. Shimada JY, Passeri JJ, Haggis LA, Callaghan OC, Lowry PA, Yanneks G, et al. Effects of losartan on left ventricular hypertrophy and fibrosis in patients with nonobstructive hypertrophic cardiomyopathy. JACC Heart Fail 2013;1:480-7.
18. Upagalawar A, Gandhi C, Balaraman R. Effect of green tea and Vitamin E combination in isoprotroen induced myocardial infarction in rats. Plant Foods Human Nutr 2009;64:75-80.

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±0.11     | 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±0.11     | 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*.
20. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin-phenol reagents. J Biol Chem 1951;193:265-75.
21. Kakkar P, Das B, Vishwanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984;21:130–2.
22. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
23. Rotruck JT, Pope AL, Ganther HE, Swasson AS, Haseman DG, Howkstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973;779:588–90.
24. Yang J, Chen NY, Xu XZ, Mou Y, Zheng RL. Alteration of RhoA prenylation ameliorates cardiac and vascular remodeling in spontaneously hypertensive rats. Cell Physiol Biochem 2016;39:229-41.
25. Al-Rasheed MN, Al-Oteibi MM, Al-Manee ZR, Al-Shareef AS, Al-Rasheed MN, Hasan HI, et al. Simvastatin prevents isoproterenol-induced cardiac hypertrophy through modulation of the JAK/STAT pathway. Drug Design Development Ther 2015; 9:3217-29.
26. Hamada M, Shigematsu Y, Ohtani T, Ikeda S. Elevated cardiac enzymes in hypertrophic cardiomyopathy patients with heart failure. J Japanese Circulation Soc 2016;80:218-26.
27. Shen XC, Qian ZY. Effects of crocetin on antioxidant enzymatic activities in cardiac hypertrophy induced by norepinephrine in rats. Pharmazie 2006;61:348-52.