

Scientific Approaches in Investigating the Protective Role of *Terminalia catappa* against Doxorubicin induced Cardiotoxicity

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Abstract

Original Research Article

The objective of the study is to evaluate the cardioprotective activity of ethanolic bark extract of *Terminalia catappa* against Doxorubicin induced toxicity in laboratory animals. *Terminalia catappa* Linn belonging to the family Combretaceae, widely distributed in India. The parts of *Terminalia catappa* plant has traditionally used for various diseases. Literatures supports various extracts of leaves and bark of the plant have been reported to have anticancer, antioxidant, hepatoprotective, anti-inflammatory, antimicrobial and nephroprotective. The aim of the present study was to investigate the cardioprotective effect of ethanolic bark extract of *Terminalia catappa* against Doxorubicin induced toxicity in rats. The rats were divided into 5 groups of 6 animals each. Group I served as normal control administered with vehicle, Group II served as toxic control, administered with Doxorubicin (15mg/kg, bw) intraperitoneally as single dose on 7th day. Group III served as reference control, administered orally with Vitamin C (50 mg/kg, bw), Group IV and V administered orally with 200 and 400mg/kg of ethanolic bark extract of *Terminalia catappa* respectively. All the test drugs were administered once daily for 15 days by suspending in vehicle. On 16th day blood was collected under Pentobarbitone (45mg/kg, i.p) anaesthesia. The separated plasma was subjected to various biochemical tests like estimation of cardiac biomarkers Creatinine Phosphokinase and Lactate Dehydrogenase. The animals were sacrificed and heart tissue homogenate was subjected for the estimation Superoxide dismutase and Catalase. The results showed that, animals treated with 200 and 400 mg/kg of *Terminalia catappa* bark extract significantly decreased the cardiac biomarker enzymes Creatinine Phosphokinase and Lactate Dehydrogenase and increased the levels of Superoxide dismutase and Catalase. From the results it was concluded that, ethanolic bark extract of *Terminalia catappa* exhibits cardioprotective activity against Doxorubicin induced cardiotoxicity in rats.

Keywords: Terminalia catappa, Cardioprotective, Doxorubicin, Creatinine Phosphokinase, Lactate Dehydrogenase, Superoxide dismutase and Catalase.

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INTRODUCTION

The cardiovascular diseases is considered as one of the major cause of morbidity and mortality in developed countries over the last several decades, and developing countries are rapidly catching up with this epidemic [1]. The underlying pathology is vascular disease, resulting in coronary artery disease, cerebrovascular disease, and peripheral vascular disease, and the subsequent development of heart failure and cardiac arrhythmias. The major risk factors for these disorders were recognized over many years, and they include high levels of low-density lipoprotein cholesterol, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, insufficient consumption of fruits and vegetables, excess consumption of alcohol, and lack of regular physical activity. The UN, alarmed by the increasing burden of cardiovascular diseases

among non-communicable diseases in low-income and middle-income countries compared with high-income countries, acknowledged in 2012 that the rising burden of cardiovascular diseases are one of the major threats to sustainable development in the 21st century [2]. The use of herbal medicines, one of the main therapeutic approaches of complementary and alternative medicine, can be tracked back thousands of years ago in the East. Currently, there is a recent resurgence of the use of herbal medicines in popularity among patients in the West. With increasing enhancement of people's awareness of self-care and concerning on the inevitable adverse effects of conventional medicine, herbal medicines are favored by people with cardiovascular diseases all over the world for their unique advantages in preventing and curing diseases, rehabilitation, and health care [3]. There is growing evidence showing that

many herbal medicines and their active ingredients contribute to the standard therapy for cardiovascular diseases for example, aspirin, digitalis, and reserpine [4]. Despite enormous interests in the medicinal uses by consumers, there is still a great deal of confusion and misunderstanding about their identification, effectiveness, pharmacology, toxicology, and herb-drug interaction to science world [5]. Therefore, the role of herbal medicines in cardiovascular diseases still needs more scientific and clinical data proving their efficacy and safety.

Terminalia catappa Linn belonging to the family Combretaceae, is distributed in the warmer parts of India. It is also known as Indian almond, Malabar almond, and tropical almond [6]. The various extracts of leaves and bark of the plant have been reported to be anticancer, antioxidant [7], hepatoprotective [8], anti-inflammatory [8], antimicrobial [9] and nephroprotective [10]. The fallen leaves of *Terminalia catappa* have been used in the management of sickle cell disorders [11]. The moderate consumption of the seed kernel is useful in the treatment of men with sexual dysfunctions, primarily from premature ejaculation [12]. Meanwhile, *Terminalia catappa* Linn, a medium sized tree has been identified with potent antioxidant activity which has been exploited as curative agents against a number of pathological conditions. Its fruits have been used for the treatment of asthma and diabetes [13]. Nuts are very nutritious and contain a significant amount of high-quality proteins and vital minerals [14]. The bark of *Terminalia catappa* reported to have diuretic and cardio-tonic property [15]. In order to validate its traditional claim, the study was design to evaluate the protective effect of ethanolic bark extract of *Terminalia catappa* against Doxorubicin induced cardiotoxicity.

MATERIALS AND METHODS

Collection and Authentication

The barks of *Terminalia catappa* was collected from outskirts of Pondicherry. The plant was identified as *Terminalia catappa* and authenticated by the botanist, Botanical Survey of India, Agricultural University, Coimbatore. The voucher specimen (BSI/SRC/11/72/2017-18/Sci/01342) has been deposited in the herbarium for future reference.

Extraction

The collected *Terminalia catappa* bark was washed, dried at shade at room temperature, pulverized by a mechanical grinder and sieved through 40 meshes. The powdered materials were extracted with 70% ethanol by cold maceration process. The extract was concentrated under reduced pressure using rotary evaporator. The ethanol free semi-solid mass thus obtained was stored in air-tight container and used for further studies.

Animals

Healthy male Sprague – Dawley (SD) rats weighing between 180 - 220 gm were used for this study. The animals were obtained from animal house, Kerala Veterinary and Animal Science University, Mannuthy, Kerala. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of $24 \pm 2^\circ\text{C}$ and relative humidity of 30–70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines.

Pharmacological Activity

Cardioprotective Activity [16]

The rats were divided into 5 groups of 6 animals each. Group I served as normal control administered with 1ml/kg of 0.1% CMC solution for 15 days. Group II served as toxic control, administered with Doxorubicin (15mg/kg, bw) intraperitoneally as single dose on 7th day. Group III served as reference control, administered orally with Vitamin C (50 mg/kg, bw), once daily for 15 days. Group IV and V administered orally with 200 and 400mg/kg of ethanolic bark extract of *Terminalia catappa* respectively. Group III, IV and V along with test drugs, the animals administered with Doxorubicin (15mg/kg) as mentioned earlier. All the test drugs were administered orally by suspending in 0.1% CMC solution. On 16th day blood was collected in heparinized tube by retro orbital sinus puncture, under Pentobarbitone (45mg/kg, i.p) anaesthesia. The collected blood samples were centrifuged for 10 minutes at 2000 r.p.m. and plasma was separated. The separated plasma was subjected to various biochemical tests like estimation of cardiac biomarkers Creatinine Phosphokinase [17] and Lactate Dehydrogenase [18]. After blood collection, the animals were sacrificed by excess Pentobarbitone and heart tissue was quickly dissected out and washed in ice cold saline. A weighed quantity of each heart was taken from all the groups and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation Superoxide dismutase [19] and Catalase [20].

Statistical analysis

Results were expressed as mean \pm SEM. The data were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's 't' test using Graph Pad version 3. P values < 0.05 were considered as significant.

RESULTS

Table-1: Effect of *Terminalia catappa* bark extract on Doxorubicin induced toxicity in rats

Drug Treatment	Plasma		Cardiac Tissue Homogenate	
	Creatinine Phosphokinase (IU/L)	Lactate Dehydrogenase (IU/L)	Superoxide Dismutase (Units/mg of Protein)	Catalase (Units/mg of Protein)
Vehicle Control (0.1% CMC)	140.55 ±6.70	148.66 ±6.89	37.78 ±2.26	61.78 ±3.44
Doxorubicin (15mg/kg)	287.35 ±4.98	257.20 ±7.20	21.80 ±1.84	32.22 ±2.90
Vitamin C (50mg/kg)	145.90 ±7.22***	152.35 ±5.50***	33.22 ±2.06***	58.98 ±3.44***
<i>Terminalia Catappa</i> Bark (200mg/kg)	150.22 ±6.04***	159.73 ±4.33***	26.26 ±1.98***	50.53 ±4.60***
<i>Terminalia Catappa</i> Bark (400mg/kg)	147.24 ±7.90***	155.26 ±5.32***	30.70 ±2.65***	55.45 ±3.65***

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Induced Control

Ethanollic bark extract of *Terminalia catappa* was studied for its cardioprotective activity against Doxorubicin induced cardiotoxicity in rats and the results were shown on table 1. The cardioprotective effect of *Terminalia catappa* was evaluated by assessing cardiac biomarker enzymes Creatinine Phosphokinase, Lactate Dehydrogenase and the levels of Superoxide dismutase and Catalase in cardiac tissue homogenate against the Doxorubicin induced toxicity in rats. In Doxorubicin intoxicated rats, the cardiac enzyme levels (Creatinine Phosphokinase and Lactate Dehydrogenase) were increased and the free radical enzymes (Superoxide dismutase and Catalase) were decreased, when compared to the levels of vehicle control animals, which confirms the damage of cardiac tissues. Vitamin C, the reference control, significantly reduced (P<0.001) both the cardiac biomarker enzymes Creatinine Phosphokinase and Lactate Dehydrogenase and significantly increased (P<0.001) the levels of Superoxide dismutase and CAT. The animals treated with 200 and 400 mg/kg of *Terminalia catappa* bark extract significantly decreased (P<0.001) the cardiac biomarker enzymes Creatinine Phosphokinase and Lactate. Dehydrogenase and increased (P<0.001) the levels of Superoxide dismutase and Catalase. The results showed that, ethanolic bark extract of *Terminalia catappa* exhibits cardioprotective activity against Doxorubicin induced cardiotoxicity in rats.

CONCLUSION

From the result, it was concluded that, the ethanolic bark extract of *Terminalia catappa* exhibited cardioprotective activity against, Doxorubicin induced cardiotoxicity in rats. Its protective activity may be due to its antioxidant property. Further detailed study may perform on *Terminalia catappa* by isolating the active principle and with other *in-vivo* models to confirm its mechanism of action.

REFERENCES

- Ralston J, Reddy KS, Fuster V, Narula J. Cardiovascular diseases on the global agenda: the United Nations high level meeting, sustainable development goals, and the way forward. *Global heart*. 2016 Dec 1;11(4):375-9.
- Joshi P, Islam S, Pais P, Reddy S, Dorairaj P, Kazmi K, Pandey MR, Haque S, Mendis S, Rangarajan S, Yusuf S. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. *Jama*. 2007 Jan 17;297(3):286-94.
- Tachjian A, Maria V, Jahangir A. Use of herbal products and potential interactions in patients with cardiovascular diseases. *Journal of the American College of Cardiology*. 2010 Feb 9;55(6):515-25.
- Xiong X, Yang X, Liu Y, Zhang Y, Wang P, Wang J. Chinese herbal formulas for treating hypertension in traditional Chinese medicine: perspective of modern science. *Hypertension Research*. 2013 Jul;36(7):570.
- Izzo AA, Di Carlo G, Borrelli F, Ernst E. Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. *International journal of cardiology*. 2005 Jan 19;98(1):1-4.
- Nadkarni KM. *Indian Materia Medica*. 4th ed. Bombay: Popular Prakashn. 1976; 1205.
- Masuda T, Yonemori S, Oyama Y, Takeda Y, Tanaka T, Andoh T, Shinohara A, Nakata M. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. *Journal of Agricultural and Food chemistry*. 1999 Apr 19;47(4):1749-54.
- Pawar SP, Pal SC, Kasture VS. Anti-inflammatory activity of *Terminalia catappa* Linn. *Pharmacy focus in the new millennium abstracts*. 1997;110.
- Neelavathi P, Venkatalakshmi P, Brindha P. Antibacterial activities of aqueous and ethanolic extracts of *Terminalia catappa* leaves and bark against some pathogenic bacteria. *Int. J. Pharm. Pharm. Sci*. 2013;5(1):114-20.

10. Vijayaprakash S, Langeswaran K, Jagadeesan AJ, Revathy R, Balasubramanian MP. Protective efficacy of Terminalia catappa L. leaves against lead induced nephrotoxicity in experimental rats. *Int J Pharm Pharm Sci.* 2012;4(Suppl 3):454-8.
11. Tan GT, Pezzuto JM, Kinghorn AD, Hughes SH. Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. *Journal of natural products.* 1991 Jan;54(1):143-54.
12. Ratnasooriya WD, Dharmasiri MG. Effects of Terminalia catappa seeds on sexual behaviour and fertility of male rats. *Asian Journal of Andrology.* 2000 Sep 1;2(3):213-20.
13. Teotia S, Singh M. Hypoglycemic effect of Prunus amygdalus seeds in albino rabbits. *Indian journal of experimental biology.* 1997 Mar;35:295-6.
14. Eley G. Wild fruits and Nuts. Yorkshire: EP Publishing Ltd. Ellman, G.L. Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics.* 1976; 82, 70.
15. Parrotta JA. Healing plants of peninsular India. CABI publishing. 2001.
16. Prabhu S, Jainu M, Sabitha KE, Devi CS. Cardioprotective effect of Mangiferin on Isoproterenol induced Myocardial Infarction in Rat. *Indian Journal of Experimental Pharmacology.* 2006; 44, 209-215.
17. Henry RJ, Chiamori N, Golub OJ, Berkman S. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase. *American journal of clinical pathology.* 1960 Oct 1;34(4_ts):381-98.
18. Ellman GL. Determination of sulfhydryl group. *Arch. Biochem. Biophys.* 1959;82(1):70-7.
19. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological chemistry.* 1972;247(10):3170-5.
20. Clairborne A. Catalase activity. In *Handbook of methods for oxygen radical research*, edited by RA Greenwald, CRC Press, Boca Raton. 1985; 283.