

ORIGINAL RESEARCH ARTICLE

Cardioprotective Potential of *Buchanania axillaris* on Doxorubicin Induced Cardiotoxicity in albino Rats

B. Santhosh Kumar¹, G. Arumugam², A. Mohammed Sadiq¹, M.Nagalingam¹, M.Gopikrishnan³, U. Kanagavalli^{*1}

¹Department of Biochemistry, Adiparasakthi College of Arts and Science, Kalavai-632 506, Vellore District, Tamilnadu, India

²Department of Zoology, Thiruvalluvar University, Serkadu, Vellore-632115, Vellore District, Tamilnadu, India

³Department of Biochemistry, University of Madras, Guindy Campus, Chennai-600 025

Received 22 Dec 2013; Revised 30 Mar 2014; Accepted 11 Apr 2014

ABSTRACT

Aim of the present study to assess the cardioprotective potential of the ethanol extract of *Buchanania axillaris* against Doxorubicin induced cardiotoxicity in albino rats. The animals were divided in to four groups. The biochemical estimation was carried out in normal group as well as experimental animals. Histology profile was assessed in normal animal as well as experimental rats. The AST level was elevated significantly in animals treated doxorubicin as compared to Group I. the level was reduced in Group III, IV. This finding shows the strongest role of *Buchanania axillaris* as a cardioprotective agent against Doxorubicin induced cardiotoxicity.

Key words: Doxorubicin (DOX), Heart, *Buchanania axillaris*, Medicinal plant.

1. INTRODUCTION

Globally cardiovascular diseases (CVD) constitute a leading cause of mortality. Developing countries like India are also struggling to manage the impact of CVD along with the growing burden of obesity, Type II diabetes and hypertension [1]. Heart disease in India occurs 10 to 15 years earlier than in the west. One fifth of the deaths in India are from coronary heart disease (CHD). By the year 2020, it will account for one third of the deaths. Current projections suggest that by the year 2020, India will have the largest CVD burden in the world. Cardiotoxicity is a major problem with hundreds of pharmaceutical agents, industrial chemicals and naturally occurring products. In the pharmaceutical sector, several compounds have been shown to lengthen cardiac repolarization, leading to arrhythmia and its clinical manifestation, One such drug is doxorubicin (DOX) used to treat cancer, which can cause cardiac disease [2]. The mechanisms proposed that doxorubicin bound with ferric iron to induce the production of reactive oxygen species that leads to causing impairment of cell functioning and cytolysis and also bound β -glycoprotein induce the production of caspase [3] and apoptosome that

cause DNA damage. DNA damage in proliferative cells activates a pathway that arrest cell division to allow either DNA repair or the induction of cell death by apoptosis [4].

Presently the medical fraternity and the patients have increasingly started using plant to overcome various illnesses and suffering mainly to obviate the profound side effects encountered in usage of modern drugs [5]. They safely interact with free radicals and terminate the chain reaction before vital molecules are damaged [6]. The prophylactic and therapeutic effect of many plant foods and extracts in reducing cardiovascular disease has been reviewed [7]. As few systematic scientific studies are currently available, these medicinal plants need to be investigated scientifically.

Buchanania axillaris (Anacardiaceae) is a traditional medicinal plant distributed in India and other Asian countries. The leaf extract has been reported to possess anti-inflammatory (Madhavachetty et al., 2008). The aerial parts are used to cure itch of the skin and to remove blemishes from the face. The kernels are used in Indian medicine as a brain tonic. The gum is anti-

diahoreal and it's used internally for rheumatism (Khare, 2004). In addition the ethanolic extract of the aerial parts showed CNS depressant activity in mice. Further, the leaves are reported to be cooling, digestive, expectorant, purgative, depurative and aphrodisiac and are useful in hyperdipsia, burning sensation, cough, bronchitis, dyspepsia, leprosy and constipation [8]. In the present study was undertaken to investigate the cardioprotective effects of the methanolic leaf extract of leaf extract of *Buchanania axillaris* on DOX induced cardiotoxicity in rats.

2. Materials and methods

2.1 Plant Materials

The leaves of *Buchanania axillaries* were collected during the month of February-August, (2012) from in and around Vellore District, Tamilnadu, India. The plant material was cleaned with distilled water and shade dried at room temperature. Leaves of *Buchanania axillaries* were collected identified and authenticated by a Botanist, Dr.C.Madhavachetty, Tirupathi university, Tirupathi, India.



(*Buchanania axillaries*)

2.2 Preparation of Plant extracts

Leaves were cleaned with water and dried in the *Buchanania axillaries* until a constant weight was obtained. Then it was powdered using a mechanical grinder to obtain a coarse powder. Equal quantity of powder was passed through 40 mesh sieve and extracted with ethanol (90% v/v) in Soxhlet apparatus at 60°C. The solvent was completely removed by rotary vacuum evaporator. The extract was freeze-dried and stored in vacuum desiccators.

2.3 Animals

Adult Wistar albino rats weighing around 200-225 g were used. These were procured from Tamilnadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India. These

animals were kept in polypropylene cages (three in each cage) at an ambient temperature of 25±2°C with 55-65% relative humidity. 12 hrs light and dark schedules were maintained in the animal house till the animals were acclimatized to the laboratory conditions, they were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, Karnataka, India). They had free access to water. The experiments were designed and conducted in accordance with the institutional guidelines.

2.4 Drugs

Doxorubicins, *Buchanania axillaries*, Verapamil (Drug), were purchased from Medaux International Life Saving Park, Chennai.

2.5 Experimental Protocol

- Group I** : Normal Rat.
Group II : Group II was treated with single dose of Doxorubicin (10 mg/kg.i.v) in normal saline and animals were sacrificed 48 hours after the administration of doxorubicin.
Group III : BA administrated for continuous 9 days (500 mg/ kg) and on 10th day DOX (10 mg/kg body weight) intravenous administrated.
Group IV : Verapamil (i.v) (5 Micro mole/kg) was given to DOX induced rat.

2.6 Estimation of Biochemical

The activities of aspartate aminotransferase (AST) were estimated by the method of [9]. The levels of lactate dehydrogenase (LDH) by the method of [10] and creatine phosphokinase (CPK) by the method of [11] were determined, using commercially available kits.

2.7 Histological studies in heart

The animals were sacrificed, and the rat was cut open to remove the heart. Then, 5mm thick pieces of the heart were fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid 0 for 12 hours and then embedded in paraffin, using conventional methods and cut into 5mm thick sections and stained, using haematoxylin-eosin dye and finally mounted in diphenyixylene. Then the sections were observed under microscope for histopathological changes in heart architecture, and their photo micrographs were taken [12].

2.9 Statistical analysis

The results were expressed in mean ± standard deviation. Statistical analysis was carried out by

using one way ANOVA as in standard statistical software package of social science (SPSS).

3. RESULTS

To study the cardioprotective effect of the ethanol leaf extract of *Buchanania axillaries* on the Doxorubicin induced cardiotoxicity in rats. The activity levels of AST, LDH and CPK were significantly elevated in DOX induced cardiotoxicity in rat when compared to that of Group-I. DOX induced cardiotoxicity rats showed a significant elevated in sodium and along with depleted level of potassium as compared to that of Group-I.

The ethanol leaf extract of *Buchanania axillaries* later supplemented with DOX induced rat have showed a significant depletion in the activity levels of AST, LDH, CPK, Sodium and along with aggrandized level of potassium as compared to normal group (**Table 1 & Fig 1**). All the parameters studied with *Buchanania axillaris* 500mg/kg body weight revealed that the higher activity. The effect of *Buchanania axillaris* was

more effective than Verapamil (Drug). Histological changes in response to Doxorubicin administration where prominent in rats, as evident in significant vacuole formation in cardiomyocytes shows characteristics patterns of structural damage. Doxorubicin treatment results in myofibrillar loss and subsarcolemmal and bleb formation. Spaces resulting from structural loss coalesce into vacuoles visible in light microscope and in emerging vacuoles represent the end result of cytological damage caused by free radicals cascades. Vacuoles are a reliable method of evaluating cellular damage since it is quantifiable. Doxorubicin treatment induced abundant macro vacuoles formation in all cardiac cells exposed to the agent. Co-treatment with *Buchanania axillaris* 500 mg/kg total dose resulted in smaller and less numerous vacuoles. The histological finding of the present study revealed that the biochemical marker evidence of Doxorubicin producing free radicals is controlled by the antioxidants which are generated by *Buchanania axillaris*.

Table 1: The effect of ethanolic leaf extract of BA on biochemical markers in DOX evoked cardiotoxicity rats

Parameters	Group-I	Group-II	Group-III	Group-IV
AST	20.33 ± 12.62	42.50 ± 12.52*	34.00 ± 5.54*	35.00 ± 3.46*
LDH	1.51 ± 0.80	1.94 ± 0.7*	1.79 ± 0.36*	1.77 ± 0.72*
CPK	65.83 ± 14.57	85.43 ± 15.33*	62.30 ± 11.83*	60.32 ± 10.53*
Sodium (Na ⁺)	1.39 ± 0.82	1.61 ± 0.40*	1.46 ± 0.41*	1.45 ± 0.49*
Potassium (K ⁺)	4.10 ± 0.40	3.03 ± 0.43*	4.01 ± 0.52*	3.90 ± 0.52*

Values are given as mean ± S.D from six rats in each group. *P < 0.05

Histological slides

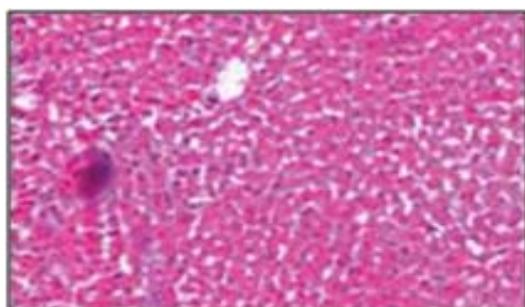


Plate: 1 (Normal)

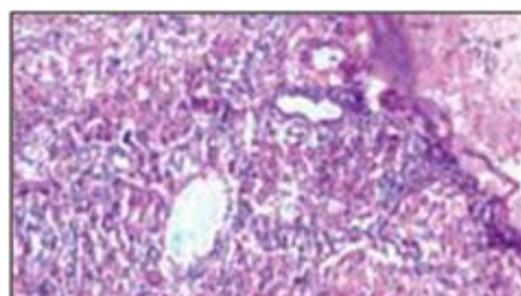


Plate: 2 (DOX)

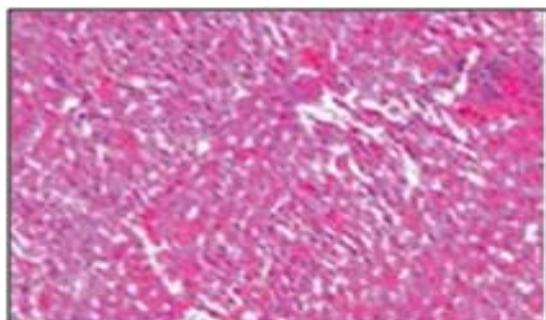


Plate: 3 (Dox + BA)

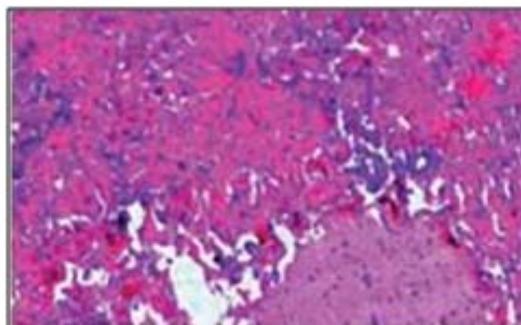


Plate: 4 (Dox + verapamil)

4. DISCUSSION

In the present study entails the cardioprotective activity of *Buchanania axillaris* (500 mg/kg body

weight) against DOX evoked cardiotoxicity in rats. In this current study, the activity levels of

AST, LDH, CPK were significantly elevated in DOX induced cardiotoxicity rats. The heart tissue damage induced by DOX in rats was indicated by the elevated levels of Serum cardiac markers. So many researchers doing experimental evidences indicated that DOX induced oxidative stress is due to the generation of free radicals in the heart tissue^[13]. The reactive oxygen species (ROS) generated tend to cause cellular damage^[14], resulting in heart tissue injury, hence therefore elevation in cardiac markers. However, the supplement with ethanolic leaf extract of *Buchanania axillaris* brought to treatment group-III were significantly depleted the activity levels of AST, CPK, and LDH. Recent reports^[15], revealed that the depletion of cardiac markers *viz.*, AST, CPK and LDH with plant supplemented group animals. In the living cell, ATPases are intimately associated with the plasma membrane and participate in the energy dependent transport of sodium, potassium, magnesium and calcium translocation^[16]. An increase in sodium potassium was observed in DOX induced rats. After supplemented with plant extract showed depleted the levels of sodium and increased the level of potassium. Evidence has been provided for DOX induced cardiotoxicity^[17] as a reasonable result for sodium and potassium. The histology of normal group-I showed a regular cell distribution. Histology of the cardiac from DOX evoked animals showed the cytoplasmic vacuole formation and myofibrillar loss. After plant treatment group showed mild edema but no infraction were seen in the histological slide. The data of the present study clearly revealed that ethanolic leaf extract modulated most of the biochemical and histopathological parameters to near normal status in DOX treated rats.

In conclusion, present study was undertaken to evaluate the efficacy of ethanolic leaf extract of *Buchanania axillaris* on Doxorubicin induced rats. However, further experimental studies are needed to isolate the active/lead biomolecules from *Buchanania axillaris* and and to investigate the exact mechanism of action.

ACKNOWLEDGMENT

The authors are immense thankful to Dr. A. Mohammed Sadiq, Principal Adiparasakthi College of Arts and Science, Kalavai, Vellore, Tamilnadu for providing sophisticated lab facility to carry out the experimental work.

REFERENCES

1. Devasagayam TPA, Sainis KB. Immune system and antioxidants, especially those

- derived from Indian Medical plants. Indian J Expt Biol. 2001; 40: 639 -655.
2. Jaenke RS, Fajardo LF. Adriamycin-induced myocardial lesions: Report of a workshop. Am J Surg Path. 1977; 1:55-60.
3. Murdoch CE, Zhang M, Cave AC, Shah AM. NADPH oxidase-dependent redox signalling in cardiac hypertrophy, remodelling and failure. Cardiovasc Res 2006; 71: 208-215.
4. L'Ecuyer T, Sanjeev S, Thomas R, Novak R, Das L, Campbell W. DNA damage is an early event in doxorubicin-induced cardiac myocyte death. Am J Physiol Heart Circ Physiol 2006; 291: 1273-1280.
5. Bhavapriya V, Kalpana S, Govindasamy S and Apparathanam T. Biochemical studies on hypoglycemic effect of Aavirai kuineer - a herbal formulation in alloxan diabetic rats. Ind. J. Exp. Bio. 2001; 39(9):925-28.
6. Chitra K and Pillai M. Guest editorial - Antioxidants in health. Ind. J. Physiol. Pharmacol. 2002; 46(1):1-5.
7. Walker AF. Of hearts and herbs. Biologist. 1996; 43(1): 177-180.
8. Mohamed H, Masmoudi Ons, Ellouz-Triki Yosra, Siala Rayda, Gharsallah Neji, Nasri Moncef. Chemical composition and antioxidant and radical-scavenging activities of *Periploca laevigata* root bark extracts. J. Sci Food & Agri. 2009; 89(5): 897 – 905.
9. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvuc transaminases. Am J Clin Pathol. 1957; 28: 56-63.
10. King J. The dehydrogenases or oxidoreductase. Lactate dehydrogenase. In: Nostrand, Van (Ed), Practical Clinical Enzymology, London. 1965; 106.
11. Okinaka S, Kumaggai H, Ebashi S, Sugita H, Momoi H, Toyokura Y, Fujie Y. Serum creatine phosphokinase. Activity in progressive muscular dystrophy and neuromuscular diseases. Arch Neurol. 1961; 4: 520-525.
12. Strate T, Mann O, Kleighans H, Rusani S, Schneider C, Yekebas E, Freitag M, Standi T, Bloechle C, Izbicki JR. Micro circulatory function and tissue damage is improved after the therapeutic injection of bovine hemoglobin in severe acute rodent

- pancreatitis. *Pancreas*. 2005; 30(3):254-259.
13. Koti BC, Vishwanathswamy AHM, Wagawade J, THippeswamy AHM. Cardioprotective effect of lipistat against Doxorubicin induced myocardial toxicity in albino rats. *Indian J Exper Biol*. 2009; 47: 41-46.
 14. Santos RV, Batista ML, Caperuto EC, Costa Rosa LF. Chronic supplementation of creatine and vitamins C and E increases survival and improves biochemical parameters after doxorubicin treatment in rats. *Clin. Exp. Pharmacol*. 2007; 34(12): 1294-1299.
 15. El-Sayed EM, Abd El-azeem AS, Afify AA, Shabana MH, Ahmed HH. Cardioprotective effects of *Curcuma longa* L. extracts against doxorubicin-induced cardiotoxicity in rats. *Journal of Medicinal Plants Research*. 2011; 5(17): 4049-4058.
 16. Ragavendran P, Sophia D, Arulraj C, Gopalakrishnan VK. Cardioprotective effect of aqueous, ethanol and aqueous ethanol extract of *Aerva lanata* (Linn.) against doxorubicin induced cardiomyopathy in rats. *Asian Pacific Journal of Tropical Biomedicine*. 2012; 1: S 212-218.
 17. Cardioprotective Effects of *Lagenaria siceraria* Fruit Juice on Isoproterenol-induced Myocardial Infarction in Wistar Rats: A Biochemical and Histoarchitecture Study. *Journal of Young Pharmacists*. 2011; 3 (4): 297-303.