Proceeding S.Z.P.G.M.I. Vol: 24(2): pp. 73-77, 2010.

Effect of Ficus Carica Leaf Extract on Cardiac Enzymes: A Study on Doxorubicin Induced Cardiotoxicity

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ABSTRACT

Objective: We have conducted a study to evaluate the protective effect of Ficus Carica (Anjiir) leaf extract on elevated levels of cardiac enzymes due to Doxorubicin toxicity in rats. **Material and Methods:** 30 Albino rats were divided into 3 groups of 10 each, one of which was control group. The 2nd group received intraperitoneal Doxorubicin, while the third also had oral feed of Ficus carica leaf extract following Doxorubicin injection. Blood was drawn after 10 days by cardiac puncture and serum was prepared for estimation of serum Lactate dehydrogenase (LDH), Creatinine phosphokinase (CPK), Aspartate transferase(AST), serum sodium and serum potassium levels. **Results:** Administration of Ficus carica leaf extract via oral route after the intraperitoneal injection of Doxorubicin produced significant decline in serum CPK and LDH levels with $p \leq 0.05$. **Conclusion:** Ficus Carica leaf extract has shown to possess protective effect on cardiotoxicity of Doxorubicin by decreasing the elevated levels of cardiac enzymes.

Keywords: Cardiotoxicity, Cardioprotective, Ficus Carica, Doxorubicin (DOX), Cardiac enzymes

INTRODUCTION

Cardiotoxicity caused by chemotherapeutic drugs is the most concerned adverse effect of these agents[1,2]. Doxorubicin, an anthracyclin antibiotic is being extensively used since the late 1960's for a wide range of hematological malignancies, many types of carcinomas, and soft tissue sarcomas. It can be used as a single agent or in combination with other chemotherapeutic agents[3].

Cardiotoxicity is the major and most concerned side effect of Doxorubicin, on which an ample of research work has been conducted since its discovery and use[4]. Estimates of the incidence of anthracyclin induced heart failure diagnosed within a year of treatment range from less than 5% to greater than 50% for cumulative doses of 550 to 1,000mg/m^2, respectively[5]. It has been found that cardiotoxicity can occur even after only four cycles of anthracyclin containing chemotherapy (240 mg/m^2) [6].

Doxorubicin induced cardiotoxicity can present acutely in the form of transient arrhythmias, pericarditis-myocarditis syndrome or left ventricular heart failure and is related to the cumulative anthracyclin dose[7] whereas chronic cardiotoxicity occurs in the form of cardiomyopathy which occurs within one year of treatment. Whereas the late onset cardiotoxicity usually occurs at least 1 year after the end of treatment[8].

Several hypothesis have been proposed to explain the cardiotoxicity of Doxorubicin, however free radical formation and resulting DNA or membrane damage have been suggested to play a critical role for its cardiac effects[9]. As the heart cells are rich in mitochondria and deficient of antioxidant defense system, so they are much more prone to this oxidative damage caused by free radicals[10].

Over the last two decades lot of research has been done to prevent the cardiotoxicity of

doxorubicin. In this regard many natural and synthetic pharmacological agents with antioxidant potential have been tried. Genus Ficus is an important group of trees in this regard because of its immense medicinal and religious values in many Asian countries[11]. Many species of this genus have been evaluated for their antioxidant effects.[12]

Ficus carica or 'Anjiir' is used in homeopathy for a number of medical illnesses. It is one of the only five plants mentioned in the Holy Quran, along with olives, grapes, pomegranate and dates[13]. Its antioxidant capacity has been evaluated but its cardioprotective effect is not studied. Because of this antioxidant potential of Ficus carica and other species belonging to this genus, we designed this study to evaluate its cardioprotective effect by measuring the serum levels of cardiac enzymes elevated by Dox.

MATERIALS AND METHOD

Ficus carica leaf extract

Leaves of Ficus carica were collected from Lahore region. They were identified and authenticated from herbarium maintained by the department of botany, Punjab University Lahore. Ethanolic extract was prepared in the chemistry department of PCSIR laboratories Lahore. Leaves of Ficus carica were dried in shade for three weeks. Dried leaves were crushed and then rinsed with hexane solution thoroughly for defattening and then filtered. The crushed matter was then immersed in ethanol for 48 hours. It was then filtered and the solution obtained was then added to rotary evaporator. On evaporation of Ethanol from the ethanol extract in Rotary evaporator (Labrota 401 digital. Heidolph), the residue obtained was stored in a dessicator[14]. Leave extract obtained was semisolid with greenish black colour and greasy consistency.

Animals

Male Sprague dawley albino rats, weighing 250-300g, were purchased from National Institute of Health(NIH), Islamabad. They were housed in standard polyprolene cages at controlled room temperature of 25±10c and relative humidity 60-70%. They were fed with standard laboratory diet with water ad libitum[15]. Three groups of 10 animals each were used for the experiment. Duration of study was for total of 10 days. On 11th day blood was taken by intracardiac puncture for serum enzymes analysis.

Drug administration

Group 1: (Control)

The animals in this group were given standard laboratory diet for 10 days.

Group 2:

On day 1 all the animals were injected with single intraperitoneal injection of doxorubicin in a dose of 15mg/kg[16].

Group 3:

All animals of this group received single intraperitoneal Injection of doxorubicin 15mg/kg on day 1, immediately followed by 400mg/kg of Ficus carica leave extract orally. The leave extract was given in the same dose daily for up to10 days. Required quantity of leave extract was measured on weighing scale and then dissolved in 5ml of distilled water. Solution according to weight of the animal was taken and then given via oral route with the help of nasogastric tube[17].

Methodology of blood collection

On 11th day animals were anaesthetized by using chloroform. They were then placed in dorsal recumbent position. After palpating the lower border of sternum, needle of syringe was introduced just to the left of xiphoid process blood was collected. All the syringes were labelled properly. The serum was then prepared for estimation of Creatinine phosphokinase (CPK),Lactate dehydrogenase(LDH), Aspartate transferase (AST), sodium and potassium were on chemistry analyser, in biochemistry Department of Shaikh Zayed hospital[18,19].

Statistical analysis

Data was analyzed by SPSS Version 15.0. The quantitative parameters CPK, LDH, AST, Na and K were described by using Mean±SD and comparison between three groups were made by

using ANOVA. A posthoc test tukey's HSD was used. A p value of ≤ 0.05 was considered as statistically significant.

RESULTS

In this study single intraperitoneal injection of doxorubicin (15mg/kg) in group 2 induced severe cardiac damage. This was manifested in the form of significant elevated levels of CPK and LDH ($p \leq 0.05$) in serum, whereas no significant change was seen in serum AST, sodium and potassium levels. Administration of Ficus carica leaf extract via oral route in animals of group 3, after the intraperitoneal injection of Dox produced significant decline in serum CPK and LDH levels with $p \leq 0.05$ (Table 1)

Table 1: Effect of ficus carica leaf extract on cardiac and biochemical parameters

Parameters	Control	Dox	Dox + FC leaf extract
CPK (IU/L)	608.7/18.81	830.3/32.52	479.5/31.87
LDH (IU/L)	1197.5/50.39	1245.0/33.49	726.3/50.66
AST (IU/L)	143.50/17.78	149.14/19.53	153.8/25.5
Na (mmol/l)	141.7/1.25	143.14/4.41	149.0/4.6
K (mmol/l)	4.49/0.22	4.43/0.65	4.81/0.67

DISCUSSION

Doxorubicin is a well known anthracyclin used extensively for the variety of malignancies since its discovery. But the major limitation to its use is cardiotoxicity which can occur acutely or chronically[20]. Its occurrence is greatly pronounced when dose exceeds 500mg/m^2. Over the last few years much effort has been done to find some herbal solution for anthracyclin induced cardiac damage instead of using chemical agents with more serious adverse effects. Many herbal or nutritional agents have been studied in animal models. For example a Chinese herbal medicine Schisandra Chinensis, grapefruit extracts, Ficus hispida and many other agents which have strong antioxidant potential, been tried against Dox cardiomyopathy[21,22].

We selected the Ficus carica which is found in our region and is commonly used by traditional healers for various illnesses. Although we did not evaluated its active components but biochemical studies on its sibling species showed them to be rich in tannins, flavonoid, alkaloids and polyphenols[23,24]. All of which are claimed to be excellent antioxidants. We evaluated the cardioprotective effect of its leaves. In our findings Dox increased the level of CPK by 136% in group 2 as compared to the control value which was decreased by 58% in Ficus Carica leaf extract given group. LDH level was also decreased by 56% from the elevated level of 104% by Dox. Whereas no changes were found in the level of AST, Na and Potassium. These effects of Ficus carica were comparable to other proposed cardioprotective herbs.

For example Nirengenin, which is present abundantly in grapefruit, is a flavonoid which is always claimed to be strong antioxidants. 25mg/kg of Nirengenin administration 7 days ahead of Dox is capable of reducing the CPK level by 57% from 226% increased level by Dox. Whereas reduction in LDH level was 46% from 142% elevated level[25]. These effects of Nirengenin are almost equivalent to FC in our rat model.

CONCLUSION

Our results showed the significant cardioprotective effects of Ficus carica leaf extract on cardiac damage produced by Doxorubicin, by lowering the biochemical parameters .Its active constituents should be separately evaluated to have a better idea regarding its antioxidant effect. This might be able to produce some better cardioprotective agent in patients receiving doxorubicin.

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