

Research Article

Comparison of Cardioprotective Effects of Amlodipine, Ficus Carica Leaf and Fruit Extracts on Histopathological Profile of Myocarditis Caused by Doxorubicin in Rat Model

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Abstract:

Background: Doxorubicin (DOX) is an anticancerous drug causing free radical mediated cardiotoxicity. This damage is usually treated by drugs like Amlodipine.

Objectives: To compare the protective effects of Amlodipine, Ficus carica leaf & fruit extracts on doxorubicin induced cardiotoxicity.

Methods: This study was conducted for 10 days, in Postgraduate Medical Institute (PGMI), Lahore. Total of 50 rats were divided into 5 groups with each group containing 10 rats. Group 1 (control), Group 2 (DOX 15mg/kg single intraperitoneal injection), Group 3 (DOX 15mg/kg single intraperitoneal injection + 5mg/kg/d of Amlodipine was given via NG tube daily for 10 days), Group 4 (DOX 15mg/kg single intraperitoneal injection + 400mg/kg/d of Ficus carica leaf extract orally for 10 days), Group 5 (DOX 15mg/kg single intraperitoneal injection + 400mg/kg/d of Ficus Carica fruit extract orally for 10 days).

Results: DOX produced severe (Grade 3) damage to cardiac cells. Beneficial effects produced by Ficus Carica leaf and fruit extracts were comparable to that of Amlodipine. Both the extracts have significantly reduced the Oedema, vacuolization, myocardial atrophy and degeneration in cardiac cells of rats.

Conclusion: The Ficus carica leaf and fruit extracts have potential to protect against the doxorubicin induced cardiotoxicity.

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Introduction:

Antineoplastic drugs are an integral part of oncology but a large number of these agents have adverse cardiotoxic effects. Doxorubicin is being widely used since 1960s for multiple tumours like Neuroblastomas, Gynaecological carcinomas, Wilms' tumour, Squamous cell carcinoma of the head and neck, soft tissue sarcomas, Bone sarcomas, Thyroid carcinoma, Breast carcinomas, Testicular carcinomas, Bronchogenic carcinoma, Lymphomas, Bladder carcinomas and Gastric carcinomas.^{1,2,3,4}

Doxorubicin is converted to Doxorubicinol in our body which is approximately 10 times more potent than the parent drug in inhibiting papillary muscle's isometric contraction. At the subcellular level it causes the activation of p38 MAPK (which are important cellular signalling mechanisms) leading to apoptosis.⁵ Subsequently a series of events is initiated in apoptotic cells including activation of proteases, sphingomyelinases, vesicle formation, plasma membrane bleb formation, and disruption of cytoskeletal, leading to cytoplasmic contraction, nuclear chromatin condensation, and DNA fragmentation.⁶ The oxygen and

hydroxyl radical cascade results in peroxidation of mitochondrial lipid membranes. Heart cells are inherently deficient in antioxidants and rich in mitochondria, therefore they are more sensitive to this oxidative damage⁷.

Doxorubicin induced cardiotoxicity can present acutely, even after a single dose or in the chronic form presenting after many years of stopping the treatment. Acute cardio-toxicity expresses itself as pericarditis-myocarditis syndrome or left ventricular heart failure, transient arrhythmias, and is associated with increasing anthracycline dose.⁸

Amlodipine is a third generation dihydropyridine (DHP) Calcium channel blocker (CCB) whose main mechanism of action is to inhibit the calcium influx in smooth muscles of vessels and myocardial cells which leads to lowering of peri-pheral vascular resistance and hence lowered Blood Pressure. Amlodipine is considered to be the first line antihypertensive agent based on its efficacy⁹. An Antihypertensive long term use evaluation study (VALUE) compared Amlodipine and Valsartan for their cardioprotective and antihypertensive effects because both are directly related. The results of this trial also proved that Amlodipine based therapy was remark-ably more beneficial in lowering Blood Pressure than Valsartan and other Antihypertensive agents which proves its cardioprotective potential as well.¹⁰ Another meta analysis compared amlodipine with other non CCB based antihyper-tensive regimens for their cardioprotective effects which also proved the superiority of Amlodipine in this context and in addition it also decreased the mortality rate.¹¹ However use of Amlodipine is associated with certain adverse effects which calls for searching safer plant based natural remedies.

The roots, stem, bark, leaves, latex, fruit and pulp of the *Ficus carica* plants contain variety of bioactive compounds, such as polyphenols, phenolic acids, triterpenoids, flavonoids, anthocyanins, carotenoids, glycosides, polysac-charides, reducing compounds, and vitamins K, E, and C. Most of these phytochemical compounds have strong anti-oxidant potential in the form of metal chelating, metal reducing and free radical scavenging properties^{12,13}.

Keeping in mind the above-mentioned facts, we compared the antioxidant effects of Amlodipine and *Ficus carica* on cardiac histopathology in rat model.

Methods:

After the approval of ethical board (SZMC/IRB/internal/366/2021), Male Sprague Dawley albino rats, were bought from National Institute of Health, Islamabad having weight of approx. 250-300g. Standard polypropylene cages were used to keep the rats at a room temperature of $25\pm 10^{\circ}\text{C}$ and humidity 60-70%. Standard laboratory diet along with water ad libitum was made readily available for rats.

Animals were divided into 5 groups each having 10 rats for the experiment. Fruit and leaves of *Ficus carica* were obtained from Lahore district which were verified by Department of Botany, Punjab University, Lahore. Details of groups is as under:

Group 1: (control): In this group animals were kept in standard control conditions with laboratory diet for 10 days.

Group 2: Animals in this group were given DOX 15mg/kg single intraperitoneal injection¹⁵.

Group 3: On day 1 animals in this group were given DOX 15mg/kg single intraperitoneal injection which was immediately followed by 5mg/kg dose of amlodipine orally. The amlodipine was then given daily in the same dose for total of 10 days. Amlodipine besylate (Norvasc 5mg tablets) manufactured by PARKE, DAVIS & CO Ltd was crushed, mixed in 5ml of distilled water and given to each rat via nasogastric (NG) tube.¹⁶

Group 4: On day 1, animals in this group were given DOX 15mg/kg single intraperitoneal injection immediately followed by 400mg/kg extract of *Ficus carica* leaves extract. The leave extract was given daily via NG tube for 10 days¹⁷.

Group 5: On day 1, animals in this group were given DOX 15mg/kg single intraperitoneal injection immediately followed by 400mg/kg extract of *Ficus carica* fruit extract. The fruit extract was given daily via NG tube for 10 days¹⁷.

On completion of treatment i.e on 10th day animals were sacrificed. Their body was dissected by giving a

midline incision. Heart of each rat was dissected out and then saved in 10% formalin solution in labelled containers. After this all the heart specimens were sent to Shaikh Zayed hospital Histopathology department where each of them was given a laboratory number. From the middle of the ventricular portion two sections were obtained one horizontal and one vertical. All the representative sections were processed and paraffin embedded tissue blocks were made fulfilling the international criteria¹⁸. An automatic processor was used for processing formalin fixed tissue. The processing comprised of dehydration by ethyl alcohol, clearing by xylene and impregnation by paraffin. After processing paraffin tissue blocks were made. Multiple slides with the thickness ranging from 3-5 microns stained with haematoxylin and eosin were made and examined microscopically. The slides were then examined under 40X Nikon microscope manually. Three to 4 sections were examined by two independent histopathologists.

Histopathological Criteria for Cardiotoxicity:

On light microscopy criteria for doxorubicin induced cardiotoxicity was set as follows:

Mild (Grade 1), Moderate (Grade 2), Severe (Grade 3)

1. Cytoplasmic vacuolization: Vacuole formation occurs in damaged mitochondria with loss of Cristae, swelling and coalescence because of disrupted outer membranes¹⁹.
 - **Mild:** Localized (at one point)
 - **Moderate:** Multiple sites (2 to 3 foci)
 - **Severe:** Diffusely present (more than 3 foci)
2. Tissue oedema: Accumulation of fluid within the interstitium¹⁹.
 - **Mild:** Localized (at one point)
 - **Moderate:** Multiple sites (2 to 3 foci)
 - **Severe:** Diffusely present (more than 3 foci)
3. Myocardial atrophy: Reduction in the number and size of myocardial cells²⁰.
 - **Mild:** Localized (at one point)
 - **Moderate:** Multiple sites (2 to 3 foci)
 - **Severe:** Diffusely present (more than 3 foci)
4. Myocardial degeneration: Necrosis and death of

cardiomyocytes²⁰.

- **Mild:** Localized (at one point)
- **Moderate:** Multiple sites (2 to 3 foci)
- **Severe:** Diffusely present (more than 3 foci)

Results:

Data was analysed using SPSS version 22. Sample size was determined by using software power & precision (PASS). The estimated sample was 10 per group¹⁷. Chi square test was applied to see the histopathological changes brought about by three different agents. P-value less than 0.005 was considered statistically significant.

All the animals in Group 2 had grade 3 vacuolization (Fig 2). Animals in DOX+amlodipine group had lowered degree of damage with 6 out of 10 animals showing grade 1 changes and 4 animals had normal cardiac histology. Ficus carica leaf and fruit extract group demonstrated grade 2 changes in all the animals (Table 1).

Table 1: Comparative effects of Amlodipine, Ficus carica leaf and fruit extracts on histopathological damage caused by Doxorubicin

Cardio-protective Agent	Vacuolization	Oedema	Myocardial Atrophy	Myocardial degeneration
Amlodipine	Grade1(6) Grade0(4)	Grade1(7) Grade0(3)	Grade0(10)	Grade0(10)
FC leaf extract	Grade2(10)	Grade2(10)	Grade1(8) Grade0(2)	Grade0(10)
FC fruit extract	Grade2(10)	Grade2(10)	Grade0(10)	Grade0(10)

All the animals of Group 2 have shown grade 3 oedema. Administration of amlodipine in group 3 reduced the oedema to grade 1 in 7 rats and to 0 in 3 rats. Whereas Ficus carica leaf and fruit extract have been able to reduce the oedema to grade 2 in all the animals (Table 1, Fig 4, Fig 5). Chi square value was found to be 9.80 with p value of 0.002.

All the animals in DOX group had grade 3 myocardial atrophy. All the animals in group 3 had grade 0 changes. Whereas in group 4, 8 out of 10 animals had grade 1 atrophy and 2 had grade 0 (Table 1 & fig 4). All the animals in group 5 with fruit extract did not show any

atrophic changes (Fig 5).

Regarding degenerative changes. All the animals in group 3, 4 and 5 had grade 0 changes. Chi square value was found to be 3.14 with p value of 0.077 which is statistically insignificant.

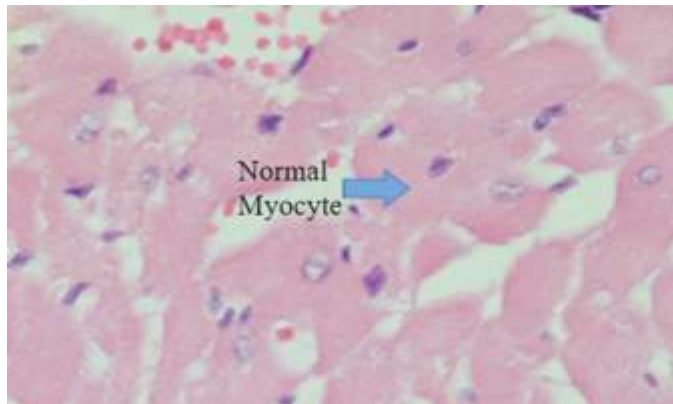


Figure 1: Group 1 (Control) showing the normal morphology of rat heart. myocardial cells are normally aligned with asymmetry and normal vascularization (H&E stain, 40X)

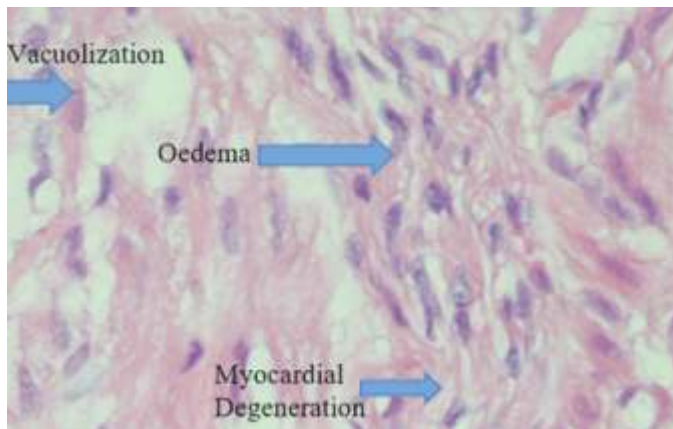


Figure 2: Group 2 (DOX) with cytoplasmic vacuolization, myocardial degeneration, myocardial atrophy, extensive oedema. (H&E stain, 40X)

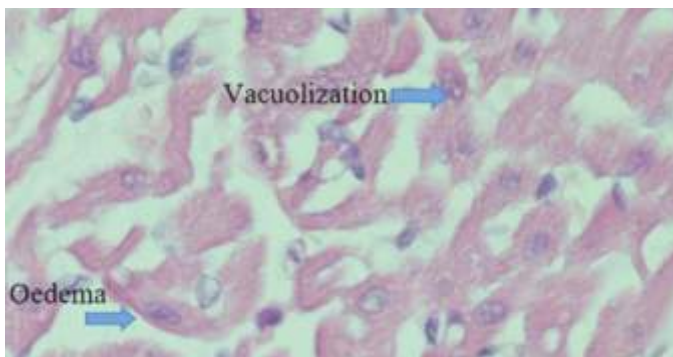


Figure 3: Group 3 (DOX+amlodipine). As compared to the Dox group cellular architecture is quite preserved with some vacuolizations and mild oedema. No degenerative or atrophic changes are evident. (H&E stain, 40X)

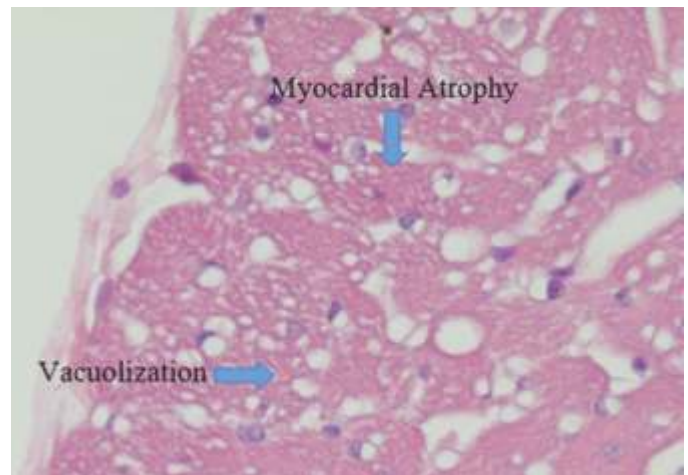


Figure 4: Group 4 (DOX+ *Ficus carica* leaf extract). Apart from mild oedema and myocardial atrophy, some vacuolizations are persisting. (H&E stain, 40X)

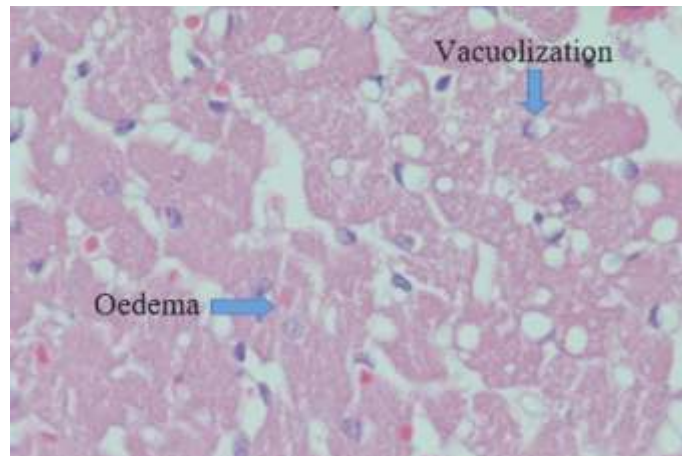


Figure 5: Group 5 (DOX+ *Ficus carica* fruit extract). Only few vacuolizations, normal cellular arrangement and traces of oedema are present. (H&E stain, 40X)

Discussion:

Results of our study showed extensive areas of damage in group 2, in the form of cytoplasmic vacuolization, tissue oedema, myocardial atrophy, and degeneration. These findings including vacuolization and oedema were significantly attenuated by amlodipine and *Ficus carica* treatment, whereas myocardial atrophy and degeneration were only present in focal areas of cardiac sections. Regarding the histopathological effects produced by amlodipine these findings are consistent with that of Yamanaka et al who showed protective effect of Amlodipine on cardiac ultrastructure. Yamanaka et al's findings were made using electron microscope and our findings were at the level of light microscopy because of limited resources; but still our

results showed the protective effect of amlodipine. These protective effects of amlodipine were more pronounced as compared to leave and fruit extracts of *Ficus carica*¹⁶. There were only few vacuolisations and mild oedema in amlodipine treated group as shown in fig 5 and 6. These protective changes brought about by amlodipine might be due to its antioxidant potential by presuming that Dox cardiotoxicity is mediated by oxidative stress²¹.

Cardioprotective effect seen by *Ficus carica* in our study is in accordance with the findings of Shah et al which proved that cardioprotective changes brought about by *Ficus carica* were due the antioxidant effects of Glucosides, proteins, glycosides, saponins, alkaloids, n-triacontanol, flavonoids, beta amyryn acetate, beta sosteriol and beta amyryndue present in this plant species²².

Another study of Allahyari et.al have seen the cardioprotective effects of *Ficus carica* leaf extract on infarcted heart and found that 70% extract could decrease the infarcted heart in rats from 80mm³ to 15mm³ of size. According to him this effect of *Ficus carica* leaf extract was because of excessive amount of flavonoids and phenolic compounds which are strong free radical scavengers.¹⁴

A study by Neha showed that 1mg of *Ficus carica* fruit extract had 10.90 µg GAE of phenolics and 2.75 µg CE/ mg of flavonoids. The Phenolics have the ability to stabilize the unpaired electron and have an ideal structure to prevent harmful oxidation through free radical-scavenging. Their antioxidant potential is more than vitamins E and C. Flavonoids are inhibitors of cell proliferation and apoptosis. This protective effect of phenolics and flavonoids of *Ficus carica* is because of free radical scavenging capacity against 2,2-diphenyl picryl hydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals^{23,24}.

Conclusion:

Ficus carica leaf and fruit extracts are enriched with flavonoids, phenolics, anthocyanins, glycosides, carotenoids, and some water-soluble vitamins. These phytochemicals have strong antioxidant activity which can be used for the management of free radical injury

by Doxorubicin. Although the cardioprotective effects produced by *Ficus carica* leaf and fruit extracts are relatively less as compared to amlodipine but they do have the ability of cardio protection. So, *Ficus carica* fruit and leaf extract alone or in combination with low dose amlodipine can provide cardio protection in DOX induced cardiotoxicity.

Ethical Approval: Given

Conflict of Interest: The authors declare no conflict of interest.

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